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Synthesis and anthelmintic activity of 7-substituted 3,4a-dimethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo-[3′,4′:4,5]furo[3,2-*b*]pyridine-6,8(7*H*)-diones

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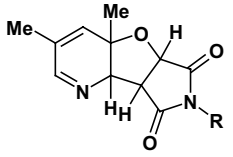
Abstract—The racemic 7-substituted 3,4a-dimethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]furo[3,2-*b*]pyridine-6,8(7*H*)-diones represent novel tricyclic compounds with strong in vivo efficacy against the parasitic nematode *Haemonchus contortus* Rudolphi in sheep. Here we report on the synthesis of tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts and describe the separation of the racemic 3,4a-dimethyl-7-ethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]furo[3,2]pyridine-6,8(7*H*)-dione into the enantiomers by HPLC. The absolute configuration of the most anthelmintically active (4a*S*,5a*S*,8a*S*,8b*R*)-enantiomer was determined by single crystal X-ray analysis using its stable (4a*S*,5a*S*,8a*S*,8b*R*)-enantiomer–$CuCl_2$ (2:1)-complex.

1. Introduction

Control of infections in human and domestic animal populations, caused by helminths, especially parasitic nematodes, remains an important global endeavour. While certain helminthiases can be treated with different groups of anthelmintics having different mode of actions[1] such as levamisol, benzimidazoles and macrocyclic lactone derivatives, the development of resistance[2] to all commercial anthelmintics underlines the need for novel specific and less toxic compounds. The search for new classes of nematicidal drugs with a different mode of action and a wide spectrum of activity for the treatment of parasitic nematodes in domestic animals plays an important role in veterinary medicine.

In the course of our recent studies to find novel classes of anthelmintic compounds related to heterocycles obtained by simple cycloaddition reaction, we have focused on tricyclic ring systems. Among these compounds some members of the class called 4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]furo[3,2-*b*]pyridine-6,8(7*H*)-dione derivatives **1**–**6** has been published.[3]



1 R = Me
2 R = Et
3 R = *c*Pr
4 R = *n*Bu
5 R = Bn
6 R = *(S)*-CHMePh

The 1,3-dipolar cycloaddition of pyridine *N*-oxides with *N*-substituted maleimides afforded the tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts **1**–**6** arising from 1,5-sigmatropic rearrangement of primary *exo*-[4+2]πcycloadducts (Scheme 1). Furthermore, the stereochemistry of the transition state was already discussed and a crystallographic evidence of the stereoselective *exo*-cycloaddition has been published.[3] We have recently disclosed our findings concerning the unexpected spin-off for antinematode treatment of novel, tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts **1**–**6**, mentioned in the title and have observed antiparasitic efficacy against animal parasites, which was previously unknown.[4]

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In this paper, we report the results of a study undertaken to investigate the preparation of these tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts **1**–**6**, physical properties, an example for the separation of enantiomers and the determination of their absolute configuration by X-ray crystallography. We also describe their activity against an gastrointestinal nematode of economic interest.

2. Chemistry

The 1,3-dipolar cycloaddition of pyridine *N*-oxides[4] with *N*-substituted maleimides[5] afforded the tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts **1**–**6**.[6] Thus, refluxing a mixture of pyridine *N*-oxides and an excess of the *N*-substituted maleimides in toluene for 10 h resulted in the formation of these rigid ring structures **1**–**6** in most cases as crystalline compounds.[7] The cycloadducts **1**–**6** were isolated in yields ranging from 9% to 63% (not optimized) after purification by column chromatography on silica gel.

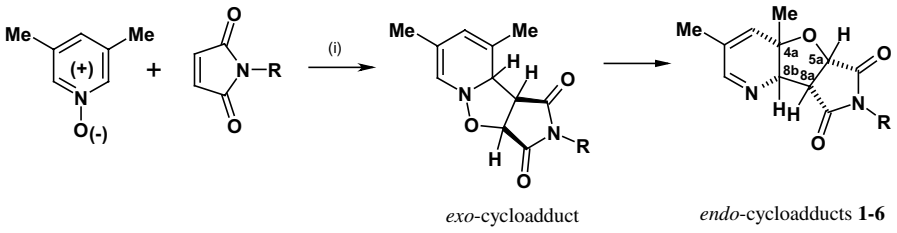
The structural assignments of the tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts **1**–**6** were based on the molecule or quasimolecule ion peaks $[M]^+$ or $[M+H]^+$ in the EI or LC–MS/APCI mass spectra and characteristic resonances in the ^1H NMR spectra assignable of the ^1H chemical shifts of methane protons H^{5a}, H^{8a} and H^{8b}. The mass spectra of **1**–**6** exhibited molecular peaks corresponding to (1:1)-cycloadducts. The evidence for stereochemistry of **1**–**6** was confirmed by literature[3] and by own NOE difference measurements of **5** in $CDCl_3$ at 400 MHz, which are not described before. Irradiation of the H^{5a} (δ 4.76 ppm) shows NOE at H^{8a} (δ 3.94 ppm) and irradiation of the H^{8b} shows strong NOEs at H^{8a} and the methyl group in 4a-position (δ 1.24 ppm), indicating that they all are situated on the same side of the central tetrahydrofuran (THF) system in **1**–**6**. The ^1H NMR spectra exhibited an AMX spin system due to the three *cis*-oriented methine protons H^{5a}, H^{8a} and H^{8b}. The H^{5a}-proton of the *endo*-cycloadduct **5**[8] appeared as a doublet (δ 4.73 ppm) with a coupling constant ($J_{5a,8a}$) of 8.0 Hz, whereas the H^{8a}- and H^{8b}-protons appeared as two double doublets (δ 3.92 and 4.22 ppm) with coupling constants ($J_{8a,5a}$/$J_{8a,8b}$ and $J_{8b,8a}$) of 8.0 Hz/9.6 Hz. The ^{13}C NMR spectrum of **5** in $CDCl_3$ at 100 MHz showed the four sp^3 carbons C_{4a} (δ 79.2 ppm), C_{5a} (δ 74.4 ppm), C_{8a} (δ 50.1 ppm) and C_{8b} (δ 65.7 ppm) for the central THF system and exhibited the rearranged tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts **1**–**6**.

For identification of the most anthelmintically active enantiomer, the resolution of both enantiomers of the racemic *endo*-cycloadduct **2** and subsequent identification of absolute configuration at C_{4a}, C_{5a}, C_{8a} and C_{8b} of its central THF system was of interest. A chiral phase HPLC assay has been developed to analyze both enantiomers of the racemic *endo*-cycloadduct **2**. The determination of *S*/*R* ratios was made by an analytical HPLC measurement on a chiral column (250 × 4.6 mm, Daicel Co.) using Chiracel-OD-H stationary phase and a mobile phase consisting of *i*-PrOH–*n*-heptane (40/60, vol./vol.; flow rate: 1.0 mL min^{-1}, detection: UV 210 nm). The ratio of enantiomers is (1:1) and both enantiomers of **2** were well separated from baseline and eluted within two peaks with retention times of 6.93 and 7.74 min, respectively. It was found, that the enantioselective analytical HPLC analysis was comparable to the following preparative HPLC method in accuracy and sensitivity. For quantitative isolation the final separation of the enantiomers of **2** was performed by HPLC on a preparative chiral column (250 × 4.6 mm, Daicel Co.) using the Chiracel-OD-H stationary phase and the mobile phase consisted of a *n*-heptane–*i*-PrOH–MeOH (87/13/3, vol./vol.; flow rate: 0.5 mL min^{-1}, detection: UV 210 nm) mixture. The purity of both enantiomers of **2** was greater than 96% as detected by integration of the responsible peak areas.

The absolute stereochemistry of the stereogenic centres in the positions 4a, 5a, 8a and 8b of the central THF system was proved directly for the most anthelmintically active enantiomer **2a** (see Table 1) by single crystal X-ray structure determination using the enantiomer **2a**–$CuCl_2$ (2:1)-complex (see Fig. 1).[9]

It is known that, in the crystalline Cu-complex of *dl*-α-aminobutyrate, the heavy Cu-atoms in the crystal can determine the phases of the structure factors in most cases.[10]

The $CuCl_2$-complex was prepared by treatment of the separated enantiomer of **2** with the metal salt $CuCl_2 \cdot 2H_2O$ in acetone at room temperature and was formed as green-yellow crystals.[11] The single crystals, which were suitable for X-ray analysis were obtained by slow evaporation of acetonitrile solution. Whereas the EI or LC mass spectra of the crystals exhibited the molecular peak of the enantiomer of **2** at *m*/*z* = 248, the APCI-MS-LOOP



Scheme 1. Reagents: (i) toluene, reflux. Synthesis of 7-substituted 3,4a-dimethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]furo[3,2-*b*]pyridine-6,8(7*H*)-diones **1**–**6**.

Table 1. In vivo anthelmintic activities against *H. contortus* in sheep and lipophilicities of 7-substituted 3,4a-dimethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]furo[3,2-*b*]pyridine-6,8(7*H*)-diones in comparison with mebendazol and ivermectin

Compound no.	Lipophilicity log *P*[a]	Anthelmintic activity *H. contortus*
Mebendazol[b]	—[c]	20[d]/3[e]
Ivermectin[b]	—[c]	0.20/3
1	<0	0.10/3
2	0.46	0.01/3
(4a*S*,5a*S*,8a*S*,8b*R*)-**2a**	0.46	0.25[f]/3
(4a*R*,5a*R*,8a*R*,8b*S*)-**2b**	0.46	0.25[f]/1
3	0.50	0.05/3
4	1.30	0.05/3
5	1.51	0.10/3
6	1.70	0.05/3

[a] Log *P*-value from HPLC (pH 2.3).
[b] Commercial product; results taken from Ref. 14.
[c] Not determined.
[d] Dose in mg test substance kg^{-1} body weight.
[e] 0 = ⩽50% egg reduction; 1 = 50–75% egg reduction; 2 = 75–95% egg reduction; 3 = ⩾95% egg reduction.
[f] Enantiomer **2a** and **2b** tested in sheep only at exemplified dose.

(neutral) showed the two peaks $[M-Cl]^+$ as well as $[M-2Cl]^+$ at *m*/*z* = 594 and *m*/*z* = 559, which are consistent with the ligand–$CuCl_2$ (2:1)-complex (calculated for $C_{26}H_{32}N_4O_6{\cdot}CuCl_2$, *m*/*z* = 629.0). The absolute configuration for the enantiomer **2a** was confirmed as *S*(C4A); *S*(C5A); *S*(C8A); *R*(C8B) (based on X-ray structure). The hexagonal Cu-complex crystallizes in the space group $P6_122$ with *Z* = 6. The Cu-atom in the centre of symmetry linked by two molecules of (4a*S*,5a*S*,8a*S*,8b*R*)-**2a** at N(1) (broken lines) and by two Cl atoms, thereby the angles of N(1)–Cu–N(1) as well as Cl–Cu–Cl are 150.2° and 133.69°, respectively. It was found that, in the (4a*S*,5a*S*,8a*S*,8b*R*)-**2a**–$CuCl_2$ (2:1)-complex, both intramolecular Cu–N(1) distances (1.98 Å) are well in accordance with stable Cu-complexes.[10] The angles of N(1)–C(2)–C(3) and C(4)–C(3)–C(2) is 125.3° and 118.7°, respectively, indicating that, in the 2,3-dihydropyridine moiety, the 1-azadiene fragment is not planar. The length of *endo*-cyclic double bonds (C=N, C=C) between N(1)–C(2) and C(3)–C(4) is 1.286 and 1.327 Å, respectively. Furthermore, the C(5A)–O(5) bond length of 1.451 Å is greater than that of the O(5)–C(4A) (1.419 Å) as described for the steric repulsion between the *N*-substituted succinimide ring and the 2,3-dihydropyridine moiety.[3c]

3. Biological evaluation

Sheep (*Ovis aries* L, Merino or Schwarzkopf breed, 25–35 kg body weight) were infected experimentally with 5000 *H. contortus* Rudolphi L_3 larvae and treated with the test substance after the end of the prepatency period of the parasite. The test compounds were administered orally in gelatine capsules. Anthelmintic effects of the test substances were measured as a function of the reduction in faecal egg count. For the purpose of counting eggs, freshly obtained faeces from experimental animals were prepared using the McMaster method as modified by Wetzel.[12] The egg counts were determined at regular intervals before and after treatment. The anthelmintic evaluation was expressed as a function of the egg reduction as follows: 3 = ⩾95%, 2 = 75–95%, 1 = 50–75% and 0 = ⩽50% egg reduction.

The tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts **1**–**6** tested in vivo were found to be fully active against the gastrointestinal nematode *H. contortus* between 0.25 and 0.01 mg kg^{-1} as outlined in Table 1.

Octanol–water partition coefficients (log *P*) were measured by an HPLC method using reverse phase columns, the general principle of which have been described elsewhere.[13]

For example, the *endo*-cycloadducts **3**, **4** and **6** were fully active against *H. contortus* at 0.05 mg kg^{-1}. The racemic

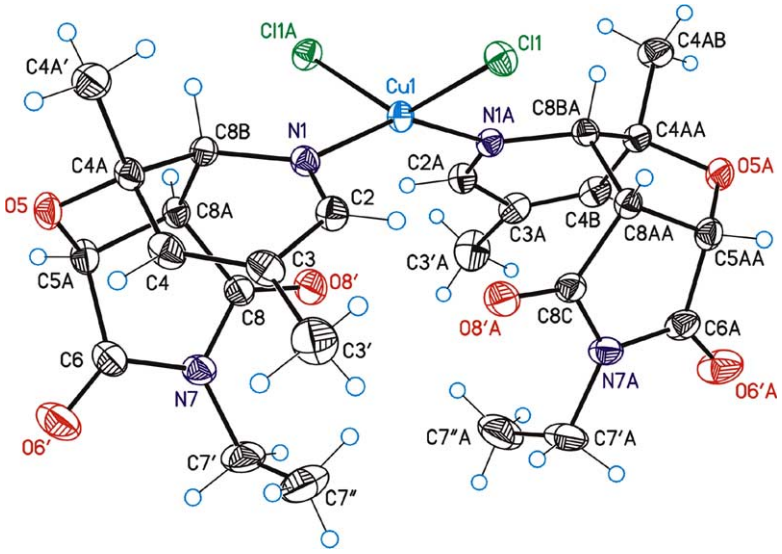


Figure 1. X-ray structure (Ortep-Plot 50%) of the (4a*S*,5a*S*,8a*S*,8b*R*)-**2a**–$CuCl_2$ (2:1)-complex.

compound **2** was the best of the tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts tested in sheep and showed full anthelmintic activity against *H. contortus* at 0.01 mg kg^{-1}. Therefore the absolute configuration of the most anthelmintically active enantiomer (4a*S*,5a*S*,8a*S*,8b*R*)-**2a** (full activity against *H. contortus* at 0.25 mg kg^{-1}), was of special interest.

The introduction of a methyl (**1**) or more lipophilic benzyl (**5**) group as *N*-substituents in 7-positon of the 3,4a-dimethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]-furo[3,2-*b*]pyridine-6,8(7*H*)-diones resulted in activities against the gastrointestinal nematode *H. contortus* at doses of 0.10 mg kg^{-1}. However, the introduction of a *cyclo*-propyl group (**3**), *n*-butyl group (**4**) or of more lipophilic and chiral (*S*)-phenethyl group (**6**), resulted in 2-fold higher anthelmintic efficacy against *H. contortus*. In the series of the *N*-substituted *endo*-cycloadducts, the *N*-ethyl derivative **2**, resulted in 5-fold higher activity against *H. contortus* compared with the derivatives **3**, **4** or **6** and gave the highest ratings against this intestinal nematode species. A comparison of the synthesized 3,4a-dimethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]furo[3,2-*b*]pyridine-6,8(7*H*)-diones **1**–**6** containing different substituents in 7-position revealed a tendency for the anthelmintic activity against *H. contortus* related to the *N*-substituent in the following order: Et ≫ *n*-Bu, *c*-Pr, (*S*)-CHMePh > Me, Bn.

In this connection, the chiral effects at the C_{4a}, C_{5a}, C_{8a} and C_{8b} atoms of the central THF system on the anthelmintic efficacy at doses of 0.25 mg kg^{-1} were examined for the separated enantiomers of the most active *N*-ethyl derivative **2**. It was shown, that the stereochemistry of the enantiomer (4a*S*,5a*S*,8a*S*,8b*R*)-**2a** seems to be attributable to the so far unknown target.

In summary, the *N*-substituted 3,4a-dimethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]furo[3,2-*b*]pyridine-6,8(7*H*)-diones **2** and **3**, which have log *P*-values up to 0.50, as well as the more lipophilic derivatives, **4** and **6**, represent the most anthelmintically active compounds against *H. contortus*. A comparison of the anthelmintic activity of the tricyclic *endo*-cycloadducts **1**–**6** against *H. contortus* at doses of 0.25 mg kg^{-1} demonstrates the strong dependence on the absolute configuration at C_{4a},C_{5a},C_{8a} and C_{8b} of the central THF system: (4a*S*,5a*S*,8a*S*,8b*R*) ≫ (4a*R*,5a*R*,8a*R*,8b*S*).

The *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts **1**–**6** represent novel tricyclic compounds with strong activities in livestock animals. Depending on the substituents in the 7-position of the *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts **1**–**6**, anthelmintic activity can be increased markedly. The racemic 3,4a-dimethyl-7-ethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]furo[3,2]pyridine-6,8(7*H*)-dione **2** was separated by analytical as well as by preparative HPLC. The absolute configuration at C_{4a}, C_{5a}, C_{8a} and C_{8b} of the central THF system by X-ray crystal structure of the $CuCl_2$-complex of the most anthelmintically active enantiomer (4a*S*,5a*S*,8a*S*,8b*R*)-**2a** was determined. It was found, that the stereochemistry of tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts **1**–**6** seems to be an important factor controlling their anthelmintic effects. Mode of action and tolerance studies have not yet been completed.

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7. All compounds gave satisfactory spectral and/or accurate mass data. Characteristical ^{1}H NMR data of the racemic tricyclic *endo*-2,3-dihydro[3,2-*b*]pyridine-type cycloadducts are given below. ^{1}H NMR (400 MHz, $CDCl_3$) **1**. δ 3.97 (H^{8a}), 4.26 (H^{8b}), 4.78 (H^{5a}); **2**. δ 3.92 (H^{8a}), 4.28 (H^{8b}), 4.75 (H^{5a}); **3**. δ 3.88 (H^{8a}), 4.26 (H^{8b}), 4.70 (H^{5a}); **4**. δ 3.95 (H^{8a}), 4.26 (H^{8b}), 4.76 (H^{5a}); **5**. δ 3.94 (H^{8a}), 4.24 (H^{8b}), 4.76 (H^{5a}); **6**. δ 3.86 (H^{8a}), 4.24 (H^{8b}), 4.67 (H^{5a}).
8. Synthesis of 7-benzyl-3,4a-dimethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]furo[3,2-*b*]pyridine-6,8(7*H*)-dione **5**. A mixture of *N*-benzyl maleimide (7.40 g; 0.04 mmol) and 3,5-dimethyl-pyridine *N*-oxide (2.40 g; 0.02 mmol) in toluene (30 mL) was heated for 10 h at reflux. The reaction mixture was allowed to cool, concentrated in vacuo and the residue purified by silica gel chromatography (cyclohexane–ethyl acetate, 1:1) to give 7-benzyl-3,4a-dimethyl-4a,5a,8a,8b-tetrahydro-6*H*-pyrrolo[3′,4′:4,5]furo[3,2-*b*]pyridine-6,8(7*H*)-dione (3.6 g, 58%). Mp: 123–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (s, 3H); 1.56 (d, 3H, J = 1.6 Hz); 3.94 (dd, $1H^{8a}$, J = 9.6, 7.9 Hz); 4.24 (dd, $1H^{8b}$, J = 9.6, 2.4 Hz); 4.57 (s, 2H); 4.76 (d, $1H^{5a}$, J = 7.9 Hz); 5.74 (dd, 1H, J = 2.4 Hz); 7.26–7.41 (2m, 5H); 7.53 (t, 1H, J = 2.4 Hz); ^{13}C NMR (400 MHz, $CDCl_3$): δ 17.8, 24.8 (CH_3), 42.0 (CH_2), 50.1, 65.7 (CH), 74.4 (OCH), 79.2 (C–CH_3), 128.4 (=C–CH_3), 131.1, 159.1 (Py–C), 127.5, 128.1, 128.7, 135.0 (Ph–C), 172.7, 173.7 (C=O). EI-MS: *m*/*e* (%): 310 (M^+).
9. Crystal data: $C_{26}H_{32}Cl_2Cu_1N_4O_6$, M_r = 631.00; hexagonal; space group $P6_122$, a = 8.50530(10) Å, c = 70.4873(14) Å, V = 4415.91(11) Å^3, Z = 6, ρ_{cal} = 1.424 mg/m^3, μ = 0.969 mm^{-1}. Data collection: The measurement was made on a Siemens P4 diffractometer equipped with a Smart 1000K CCD area detector, a Mac Science rotating anode with Mo Kα radiation, a graphite monochromator and a Siemens LT2 low

temperature device ($T = -120$ °C). Measurement range from 1.73° to 31.54°. 66,449 reflections were collected of which 4933 are unique ($R_{int} = 0.1280$). Fullsphere data collection ω and φ scans. Programs used: Data collection Smart V. 5.060 (BrukerAXS 1999), data reduction Saint + Version 6.02 (Bruker AXS 1999) and absorption correction SADABS (Bruker-Nonius 2004-1). Crystal structure solution was achieved using direct methods as implemented in SHELXTL Version 5.10 (Sheldrick, Universtität Göttingen (Germany), 1998); 4683 Fo > 4sig(Fo), 242 refined parameters, $R_1 = 0.0417$, $wR2 = 0.0997$, Goodness of fit on $F^2 = 1.225$, Flack parameter 0.02(0.01), maximum residual electron density 0.449 (−0.545) e Å^3. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number *CCDC 253072*. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1 EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

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