Stereochemistry of products of reactions between 3-diazo-naphthalene-1,2,4-trione and β-dicarbonyl compounds. Structure of ethyl 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-3-phenyl-3-oxo-propionate

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3-Hydroxy-2-[(R^1CO)(R^2CO)]C=NNH-1,4-naphthoquinones, obtained from reactions of 3-diazonaphthalene-1,2,4-trione with β-diketones, $R^1C(O)CH_2COR^2$, have been previously found to have high antibacterial activity. However, confirmation of the stereochemistry about the C=N bond could not be achieved by spectroscopic means for products having different R^1 and R^2 groups, thereby limiting the utility of the reaction. Full characterisation of the product isolated from reaction of 3-diazonaphthalene-1,2,4-trione with $PhC(O)CH_2CO_2Et$ is now reported, from a single crystal X-ray structure determination: the product, 3-hydroxy-2-[($PhCO$)($EtCO_2$)]C=NNH-1,4-naphthoquinone has a (*Z*)-stereochemistry. The *Z*-isomer is obtained rather than the *E* form due to the preferred formation of the stronger intramolecular N–H---O hydrogen-bond with the ester carbonyl oxygen rather than a weaker one with the ketone oxygen. Weaker C–H---O hydrogen bonds link the molecules into columns. It is suggested that similar *Z* geometries will arise from other $RC(O)CH_2CO_2R^i$ reactants.

Keywords: 1,4-naphthoquinones, lapachol derivatives, hydazones, antibacterial activity

Quinones have been the subject of considerable interest for many years as a result of their biological activities. It has been over 60 years since Wendel initially showed that certain 2-hydroxy-3-alkyl-naphthoquinones inhibited the growth of *Plasmodium*.[1] Further studies have proven that the toxicity of naphthoquinones to *Plasmodium sp*. is due to interaction with the mitochondrial respiratory chain.[2,3]

Among the family of naphthoquinones, lapachol, **1**, (a naturally occurring compound), and its derivatives have been particularly well investigated over the past decades for their antibacterial,[4-6] antifungal[7] and anticancer[8-12] activities. The anti-cancer activity of β-lapachone, **2**, an isomer of lapachol **1**, has also been intensely investigated.[13-16] Studies with substituted lapachol derivatives have indicated certain correlations between structure and biological activity. For example, a relationship was established between the length of the side chain at site 3 in 2-hydroxy-3-alkyl-substituted-1,4-naphthoquinones and the toxic effects on several microorganisms.[17,18] Fieser and Richardson showed that, as the alkyl side chain in hydrolapachol, **3**, is lengthened by the insertion of methylene units, the activity against *P. lophurae* in duck increases up to a C9-side chain, but then falls away.[19]

Ferreira and co-workers[20] have reported the synthesis and antibacterial activity of the 2-hydroxy-3-hydrazino-1,4-naphthoquinones, **4a–e**, obtained from 3-diazo-naphthalene-1,2,4-trione following a procedure published by Reid *et al.*,[21] see Scheme 1 and Fig. 1. Compounds **4** can be considered as analogues of the 2-hydroxy-3-alkyl-substituted-1,4-

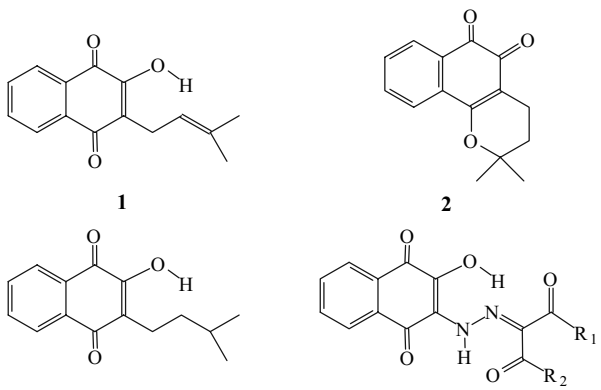
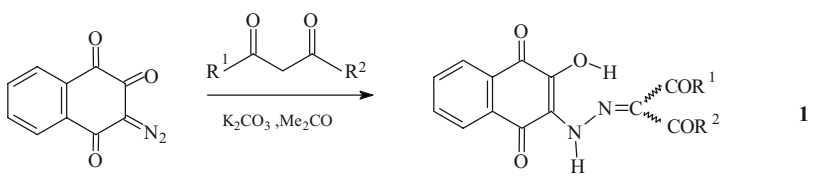


Fig. 1 Selected 1,4-naphthoquinones.



Scheme 1

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naphthoquinones. The most active of these compounds, **4b**, in preliminary susceptibility testing in discs, exhibited a higher level of antibacterial activity than lapachol, **1**. Additional studies on the minimal inhibitory concentration (MIC) for *Staphylococcus aureus* showed that **4b** has shown an activity twice that of lapachol. The stereochemistry about the hydrazone C=N bond for compounds, **4**, having different R^1 and R^2 groups, could not be established by spectroscopic means. Without confirmation of the stereochemistry, the scope of this reaction is somewhat limited to symmetrical compounds. Thus we have turned to X-ray crystallography to help solve the stereochemical problem. The unsymmetrical hydrazono compound, ethyl 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)hydrazono]-3-phenyl-3-oxo-propionate (**4f**), containing an ester and a ketone groups produced suitable crystals for the X-ray study.

Results and discussion

The isolated yields of **4a–4e**, as pure single-component solids, were reported to be in the range 55–68%,[20] while that for the new derivative, purple 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-3-phenyl-3-oxo-propinoate (**4f**), obtained from ethyl benzoylacetate, was found to be lower at 27%, after two recrystallisations. While no product balance was carried out, there were no indications of other isomeric products: starting materials had been totally consumed. No other products were isolated or identified in the reaction residue despite attempts to do so. Spectral and other data were consistent with the formula for **4f**, but could not distinguish between the possible geometric isomers. The sample of **4f** used in the crystallographic study was recrystallised from EtOH. The atom arrangement and numbering scheme are shown in Fig. 2a, while selected bond lengths and angles are listed in Table 1.

There are several possible combined stereochemical and conformational forms for **4f**, some of which are drawn in Fig. 3. The form actually determined was the [(***Z***)-**4f-I**] form, *i.e.* with a (*Z*) stereochemistry at $C_{(9)} = N_{(2)}$, see Figs 2a and 3. This geometry is cemented by the N1–H1---O6 (involving the ester group) and the O–H---N intramolecular hydrogen bonds. The corresponding **(*E*)-4f-I** form would have the ketone carbonyl oxygen atom, O3, H-bonded to NH- a less favourable situation due to the reduced H-bond ability of keto compared to ester carbonyl groups. It is noticeable that the *Z*-form adopted in the solid state is the [(***Z***)-**4f-I**] form rather than the [(***Z***)-**4f-II**] form. The (*Z*)-form, [(***Z***)-**4f-II**], would also allow the strong N1–H1---O6 intramolecular hydrogen-bonding and additionally an O2–H2---O3 intramolecular H-bond. However this is not favoured in the solid state. Instead of being involved in an intramolecular H–bond, the PhCO oxygen, O3, takes part preferentially in intermolecular hydrogen bonding. This C5–H5---$O3^{i}$ intermolecular hydrogen bond, as well as C18–H18b---$O2^{ii}$, link the molecules into columns: symmetry codes: *i*: –1/2–*x*, –1/2 + *y*, –3/2 + *z*, *ii*: –1/2–*x*, 1/2 + *y*, 1/2 + *z*, see Fig. 2b and Table 1.

Geometric parameters for the hydrogen bonds, recognised by PLATON,[22] are listed in Table 1. The intramolecular hydrogen bonds, O2–H2---N2 and O2–H2---N1, would occur irrespective of the nature of the stereochemistry at the C=N bond. In addition to the intermolecular H-bonds, there are also π–π stacking intermolecular interactions linking molecules, see Table 1. The most significant of these are shown in Fig. 2c. The stereochemistry about the C=N bond is significant while the conformation found in the solid state really has relevance only for the solid state as thermal motions and solvation effects

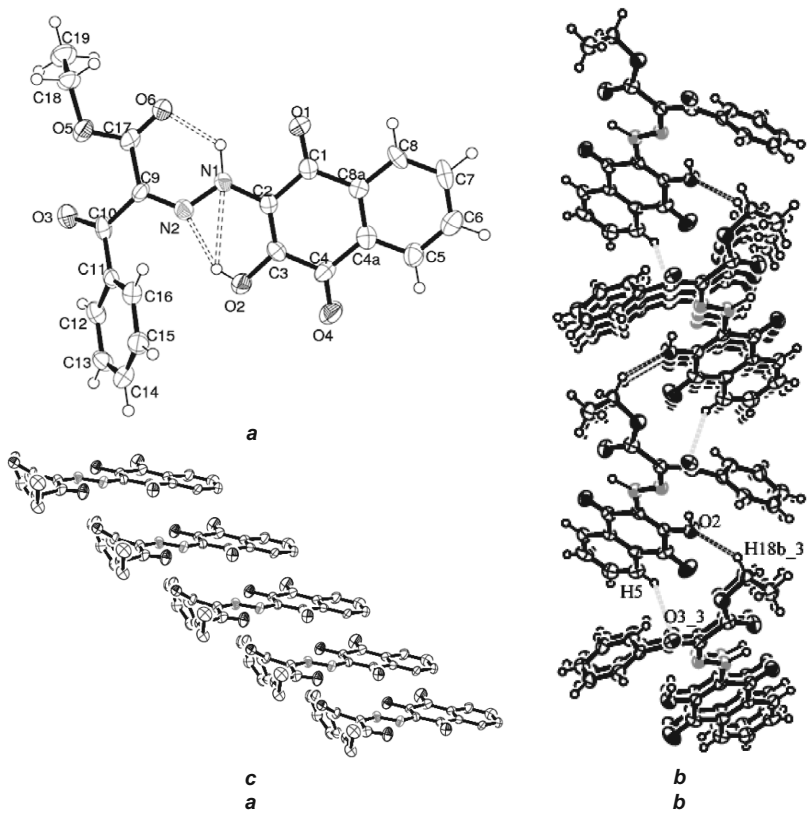


Fig. 2 (a) Atom arrangements and numbering scheme for **4f**, probability ellipsoids of atoms are drawn at the 50% level, hydrogen atoms are drawn as spheres of arbitrary radius; intramolecular H-bonds drawn as dashed lines; (b) molecules linked by intermolecular H-bonds, C5–H5---$O3^{3}$ and $C18^{3}$–$H18b^{3}$---O2; (c) molecules indicating the π–π stacking interactions involving the quinone ring [C(5)–C(8), C(8A), C(4A)] and the terminal aryl ring [C(1)–C(4), C(4A), C(8A)]: symmetry codes are listed in Table 2.

Table 1 Geometric parameters for **4f**

(a) Selected bond lengths and angles [Å,°]

O(4)–C(4)	1.211(8)	O(1)–C(1)	1.210(8)
O(2)–C(3)	1.355(8)	O(5)–C(17)	1.321(9)
O(5)–C(18)	1.443(8)	O(6)–C(17)	1.221(9)
N(1)–N(2)	1.341(7)	N(1)–C(2)	1.395(9)
N(2)–C(9)	1.300(9)	C(9)–C(10)	1.481(10)
C(10)–C(11)	1.472(10)	O(3)–C(10)	1.239(8)
C(4)–C(4A)	1.509(10)	C(8A)–C(4A)	1.405(10)
C(1)–C(8A)	1.495(10)	C(2)–C(1)	1.481(10)
C(3)–C(2)	1.361(9)	C(4)–C(3)	1.469(10)
N(2)–N(1)–C(2)	119.2(6)	C(9)–N(2)–N(1)	122.5(6)
N(2)–C(9)–C(10)	113.1(7)	C(11)–C(10)–C(9)	119.0(5)
C(3)–C(4)–C(4A)	116.1(7)	C(8A)–C(4A)–C(4)	121.4(7)
C1–C(8A)–C(4A)	120.3(7)	C(2)–C(1)–C(8A)	117.0(7)
C(3)–C(2)–C(1)	122.1(7)	C(2)–C(3)–C(4)	122.9(7)

(a) Hydrogen bonds (Å,°)

D–H---A	D–H	H---A	D---A	∠D–H---A
N1–H1---O6	1.05	1.88	2.632(6)	125
O2–H2---N1	0.82	2.45	2.923(6)	117
O2–H2---N2	0.82	1.91	2.647(7)	150
C5–H5---O3^{i}	0.95	2.39	3.207(8)	144
C18–H18B---O2^{ii}	0.99	2.56	3.183(8)	121

Symmetry codes: i = –1/2–*x*, –1/2 + *y*, –3/2 + *z*; ii = –1/2–*x*, 1/2 + *y*, 1/2 + z

(b) π–π- stacking interactions (Å,°)[a]

Cg(I)---Cg(J)	Cg··Cg	α	β	γ	Cg_{perp}	Slippage
Cg(1)---Cg(2)^{iii}	4.282(5)	1.3(4)	38.57	39.65	3.297(3)	3.348(3)
Cg(2)---Cg(1)^{iv}	4.281(5)	1.3(4)	39.65	38.57	3.347(3)	3.297(3)

[a]Cg(1) is the centroid of the ring defined by C(1)–C(4), C(4A), C(8A) and Cg(2) is the centroid of the ring defined by C(5)–C(8), C(8A), C(4A); α is the dihedral angle between the overlapping rings; β is the angle at either Cg between the vectors Cg··Cg and Cg_{perp}; Cg··Cg is the distance between the ring centroids; Cg_{perp} is the perpendicular between the ring planes and slippage, or lateral displacement, is the distance between Cg(I) and perpendicular projection of Cg(*J*) on ring I. [Symmetry codes: (iii) *x*,*y*,1 + *z* (iv) *z*, *y*,–1 + *z*]

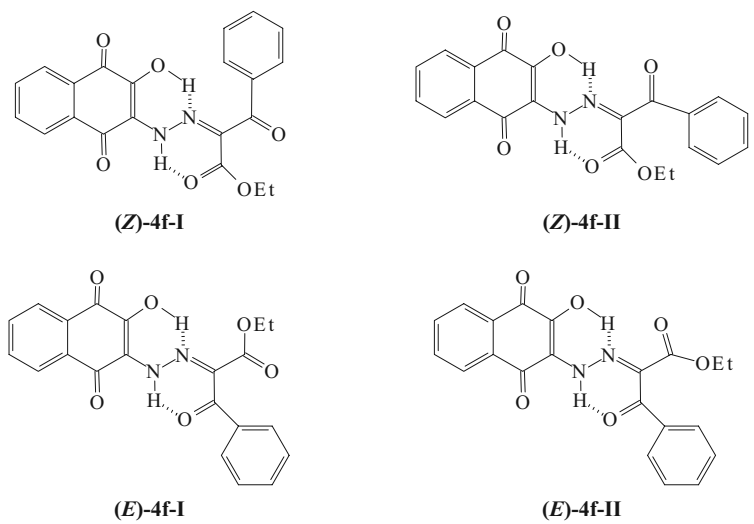


Fig. 3 Selected stereoisomers and conformers of **4f**.

are significant in solution and can affect the conformational equilibrium. The atoms, O2, C3, C2, C1, O1, C8A, C8, C7, C6, C5, C4A, C4, O4, N1, N2, C10 are essentially co-planar, with atoms O1 and O4, being the furthest out of the best plane by 0.096(4) and 0.098(4) Å, respectively. The angles between this best plane and that of the C11-C16 phenyl plane is 59.02 (12)°.

It has to be stated that **(*Z*)-4f-I** was isolated in a low yield. While no product balance was undertaken, no indication of an **(*E*)-4f** product was obtained. However, it remains a possibility that a **(*E*)-4f** stereoisomer had been formed, but had escaped detection and isolation. No indication of a tautomer of **4f** either was found.

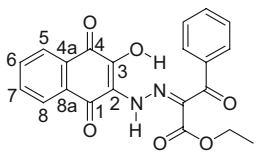
The crystallographic findings, with respect to the stereochemistry for **4f,** are clear, and suggest that similar *Z*-configurations will apply to other keto-ester compounds, such as **4b** and **4c**, shown in Fig. 1.

Experimental

NMR spectra were run in $CDCl_3$ solutions on a Varian Unity 300 MHz Plus Spectrometer, infrared spectra were recorded on a Perkin-Elmer FT-IR Spectrum One spectrophotometer calibrated relative to the 1601.8 cm^{-1} absorbance of polystyrene, thin layer chromatography on silicagel 60F-254 (5554 MERCK), 0.2 mm thick, and with spraying with aqueous ammonium sulfate (25% m/v), column chromatography on silicagel 60 (0.063–0.200 mm ref. Merck. 1.05554). Melting points were measured on Reichert micro hot stage and are uncorrected. Solvents were dried by standard methods.

Preparation of 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-3-phenyl-3-oxo-propionate (**4f**)

A mixture of ethyl benzoylacetate (0.38 mmol) and anhydrous K_2CO_3 (57 mg, 0.4 mmol) in dry acetone (15 mL) was stirred for 15 min under a nitrogen atmosphere. To this mixture was added slowly through a syringe, a solution of 3-diazo-naphthalene-1,2,4-trione (**5,** 77 mg, 0.38 mmol) in dry acetone (5 mL) during 15 min. After stirring for 2 h the reaction was acidified with 5% (v/v) HCl (40 mL), the solid material was collected by filtration gave (Z) 2-[(3-hydroxy-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)-hydrazono]-3-phenyl-3-oxo-propinoate (**4f**) as a purple solid (m.p. 178–179 °C) in 27% yield.



IR ν_{max}:1736, 1676, 1668, 1651, 1618, 1595, 1530, 1466, 1447, 1370, 1335, 1293, 1272, 1258, 1214, 1202, 1149, 959, 713 cm^{-1}. ^1H NMR (300.00 MHz, $CDCl_3$) δ 1.35 (3H, t, J = 7.1 Hz, $OCH_2C\underline{H_3}$), 4,43 (2H, q, J = 7.1 Hz, $OC\underline{H_2}CH_3$), 7.53–7.48 (2H, m, *meta*-Ph), 7.53–7.48 (1H, m, H-6); 7.69–7.75 (1H, m, *para*-Ph); 7.69–7.75 (1H, m, H-7), 7.86 (1H, d, J = 9.0 Hz, H-8); 7.93–7.97 (1H, m, *ortho*-Ph), 8.09 (1H, d, J = 9.0 Hz, H-5) ppm; ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 13.7 ($OCH_2\underline{C}H_3$), 62.3 ($O\underline{C}H_2CH_3$), 122.4 (C-2), 126.0 (C-5), 126.4 (C-8), 128.3 (C_{meta}-Ph), 128.7 (C_{ortho}-Ph), 129.3 (C_{para}-Ph), 129.5 (C=N), 130.6 (C-4a), 133.6 (C-7), 134.1 (C-6), 135.8 (C-8a), 142.5 (Ph), 144.6 (C-3), 162.4 (CO_2Et), 168.7 (C-4), 174.4 (C-1), 178.9 (PhC=O) ppm. Found: C, 64.1; H, 4.3; N, 7.2. $C_{21}H_{16}N_2O_6$ requires: C, 64.28; H, 4.11; N, 7.14%

Table 2 Crystal data and structure refinement for **4f**

Empirical formula	$C_{21}H_{16}N_2O_6$
Formula weight	392.36
Temperature	120(2) K
Wavelength	0.71073 Å
Crystal system, space group	Orthorhombic, Pna21
Unit cell dimensions	a = 20.5630(9)Å
	b = 14.633(2) Å
	c = 6.011(3) Å
Volume	1808.7(9) $Å^3$
Z	4
Density (calculated)	1.441 Mg m^{-3}
Absorption coefficient	0.107 mm^{-1}
F(000)	816
Crystal size	0.60 × 0.05 × 0.03 mm
Theta range for data collection	2.96 to 25.00 deg.
Index ranges	−17<= h<= 24;
	−17<= k<= 17; −7<= l<= 7
Reflections collected	9888
Independent reflections	1719 [R(int) = 0.1350]
Reflections observed (>2 sigma)	1050
Data Completeness	0.976
Absorption correction	None
Refinement method	Full–matrix least–squares on F^2
Data/restraints/parameters	1719/1/266
Goodness–of–fit on F@2	1.099
Final R indices [I>2 sigma(I)]	R^1 = 0.0911 wR^2 = 0.1385
R indices (all data)	R^1 = 0.1599 wR^2 = 0.1631
Absolute structure parameter	0.00 (10)
Largest diff. peak and hole	0.259 and –0.265 e $Å^3$
CCDC deposit number	676187

Crystallography

The sample was recrystallised from EtOH. Data were obtained at 120 K with Mo-K– radiation by means of the Enraf Nonius KappaCCD area detector diffractometer of the EPSRC crystallographic service, based at the University of Southampton. Data collection was carried out under the control of the program COLLECT[23] and data reduction and unit cell refinement were achieved with the COLLECT[23] and DENZO programs.[24] Correction for absorption, by comparison of the intensities of equivalent reflections, was applied using the program SADABS.[25] The program ORTEP-3 for Windows[26] was used in the preparation of the figures and SHELXL-97[27] and PLATON[22] in the calculation of molecular geometry. The structure was solved by direct methods using SHELXS-97[28] and fully refined by means of the program SHELXL-97.[27] In the final stages of refinement hydrogen atoms were introduced in calculated positions and refined with a riding model. Crystal data and structure refinement details are listed in Table 2. CCDC 676187 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif".

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