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Unexpected oxidation of a substituted benzo[*a*]phenazine: oxidative cleavage of a double bond and formation of a macrolactone

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Abstract—Peroxidation of the phenazine of β-lapachone using *m*-$ClC_6H_4CO_3H/CH_2Cl_2$ furnished a macrolactone with a rigid 10-membered ring, and the corresponding N-oxide, originating from the oxidative cleavage of the aromatic double bond at the site of fusion of the dihydropyrane moiety with the phenazine component. A third compound, a dihydrobenzophenazine-5-one, was also generated probably by hydrolysis of an intermediate epoxide-acetal or α-hydroxy-hemiacetal formed at the same site. All of the new compounds were fully characterized by X-ray diffractometry and spectroscopic methods.

The naturally occurring *ortho*-naphthoquinone β-lapachone (2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione) (**1**) exhibits a variety of biological activities including anti-cancer,[1,2] in vitro inhibition of DNA topoisomerases[3] and induction of topoisomerase IIα mediated DNA cleavage.[4] In combination with taxol, β-lapachone has shown to be highly effective against established human ovarian and prostate tumours implanted in immunosuppressed mice.[5] Several synthetic analogues of β-lapachone, all of which retain the intact *o*-quinone moiety, have been tested for anticancer activity.[4] Analogues with modifications at the site of redox activity, which may alter the redox cycling characteristics of the molecule, have, however, received less attention.[6] The syntheses of several heterocycles derived from β-lapachone and their activities against *Trypanosoma cruzi* have been reported.[7] Whilst the β-lapachone derivative 3,3-dimethyl-2,3-dihydro-3*H*-benzo[*c*]pyran[3,2-*a*]phenazine (**1**) has been known for a considerable time,[8] its ^1H NMR and UV/visible/fluorescence spectra[9,10] and X-ray crystallographic data[11] have only been described in detail recently. Benzo[*a*]-phenazine derivatives are efficient DNA intercalating ligands[12] and they also show broad-spectrum antibiotic activities.[13]

As heterocyclic aromatic N-oxides are currently being evaluated as bioreducible drugs, particularly as hypoxia-selective cytotoxins,[14] and since phenazine mono- and di-oxides are useful as broad spectrum antimicrobial agents,[13] we were interested in preparing benzo[*a*]phenazine mono- and di-N-oxides from **1** in order to study their biological activities and their chemical reactivities.

Following a simple oxidation procedure employing *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2[15], an efficient oxidation reaction took place from which it was possible to isolate and fully identify **2**, as a macrolactone of the oxecane type,[16] its N_{16}-oxide (**3**), and a dihydrobenzophenazine-5-one (**4**; Scheme 1). Compound **2** was the least polar of the oxidation products being the first to be eluted from a silica gel column and it was analysed by IR and NMR spectroscopy.[17] Elemental analysis furnished $C_{21}H_{18}N_2O_3$,[17] indicating that two oxygen atoms had been introduced into the original phenazine ($C_{21}H_{18}N_2O$). IR absorptions at 1720 and 1700 cm^{-1} suggested the presence of a ketolactone

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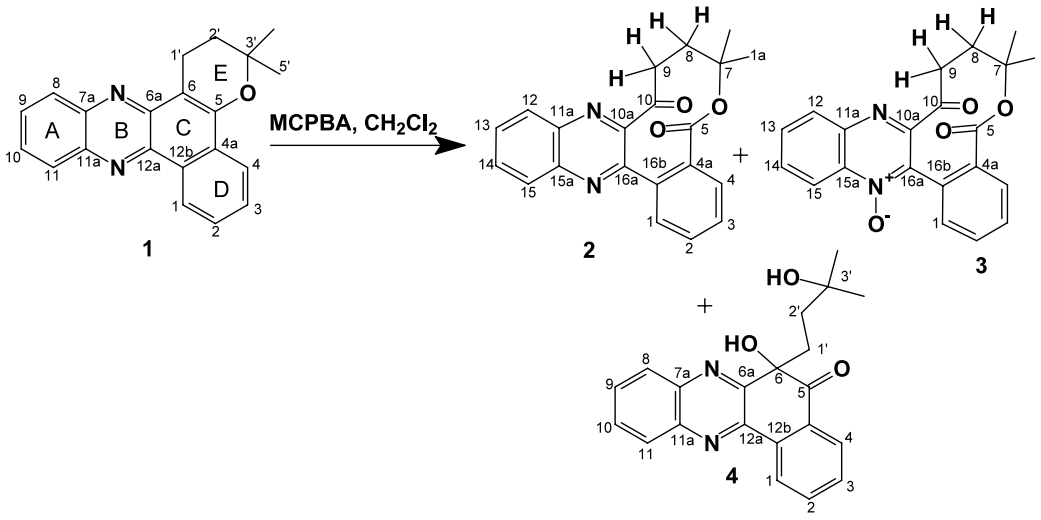

Scheme 1. The oxidation reaction of phenazine **1** giving rise to the macrolactones **2** and **3**, and the dihydrophenazone **4**.

function, and this was confirmed from the corresponding chemical shifts in the ^{13}C NMR with signals at δ 164.9 and 201.9 ppm. From these data, the N-oxidised product expected to be derived from **1** could be excluded. The significant difference in the ^{1}H NMR of **2**[17] compared to **1**,[9] represented by the absence of the downfield signal of H-8 on the phenazine convex side,[9] provides evidence for the lack of planarity between the quinoxaline and the coupled benzenic rings. However, the chemical shifts for the two methyl groups, along with the complex 4H system (4 *ddd;* δ 1.98, 2.28, 2.75, 3.8), suggest a high order of rigidity in the aliphatic carbon skeleton in the oxidised system. Structure **2** was finally confirmed by X-ray crystallography (Fig. 1).[18] The asymmetric unit consists of two independent molecules which are not related by symmetry (Fig. 1). In both molecules, no conformational differences were observed. Bond lengths and angles were in good agreement (within experimental accuracy) with the values reported in the literature.

The structural analysis of **2** was of great help in determining the structure of **3**. Comparison of the ^{1}H and ^{13}C NMR data[17,19] of the two compounds showed strong similarities with respect to the macrolactone ring C (Scheme 1), with almost the same pattern of multiplicities and of chemical shifts for the methylene signals, and an identical number of aromatic CH and of quaternary carbons. Elemental analysis of **3** produced the formula $C_{21}H_{18}N_2O_4$, showing one additional oxygen atom in relation to **2**, and suggesting the presence of an N-oxide group. X-ray diffraction data[20] allowed the determination of **3** as the N-16 oxide derivative of **2** (Fig. 2).

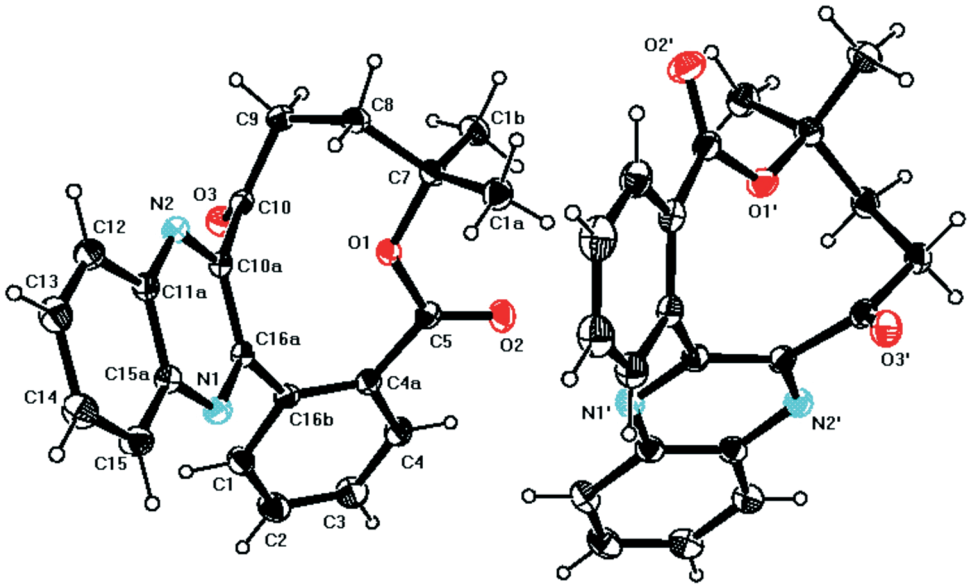

Figure 1. The structure of compound **2** showing 50% probability displacement ellipsoids and the atom-numbering scheme (note that the H atoms have been omitted for clarity.)

The third, and most polar, compound **4** was a benzo-[*a*]phenazine-5-one derivative whose structure was fully defined from X-ray diffraction data[21–25] (Fig. 3). Nevertheless, it should be noted that the ^{1}H NMR spectrum of **4** shows identical chemical shifts for the two CH_3 groups suggesting a flexible aliphatic skeleton in the structure of this compound.[26] Indeed, the NMR spectra is representative of a typical phenazinic structure with a deprotected H-1 (δ 8.77) (Scheme 1).

The crystallographic data (excluding structure factors) for the structures described in this paper have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 199827 (for **2**), 199828 (for **3**) and 199829 (for **4**).

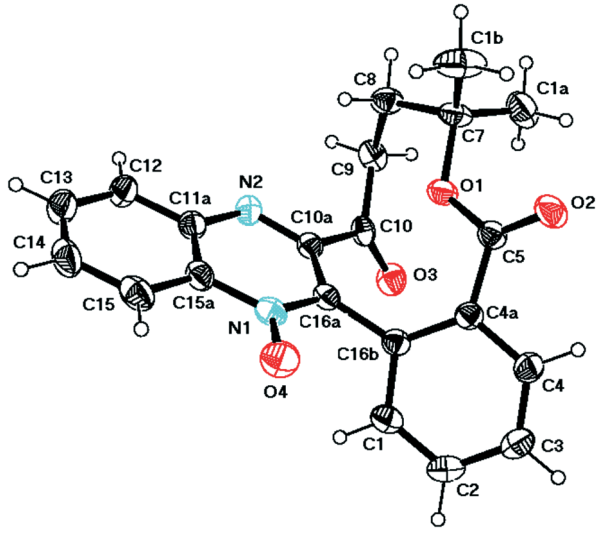

Figure 2. The structure of compound **3** showing 50% probability displacement ellipsoids and the atom-numbering scheme.

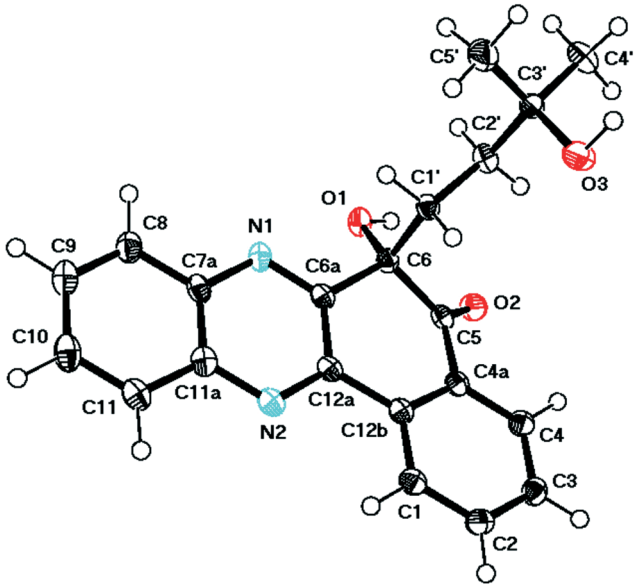

Figure 3. View of compound **4** showing 50% probability displacement ellipsoids and the atom-numbering scheme.

Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2, 1EZ, UK (fax: +44 (0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Whilst cleavage of C=C bonds can be accomplished by oxidative processes,[27] it was not expected that the cleavage of the aromatic double bond of **1** would be achieved directly in such a simple manner by MCPBA, a reagent commonly used to bring about the epoxidation of a double bond. This type of reaction is rarely observed, but may be exemplified by the cleavage of the double bond of tetrahydrochromane (an enol ether) to a middle-size cyclic ketolactone.[28,29] In this case, the postulated mechanism involves the intermediacy of an epoxy ether which is converted by MCPBA into a hydroxy per-ester, which could then react with MCPBA or with the resulting and stronger acid *m*-chlorobenzoic acid (MCBA).[29]

To our knowledge, the present report represents an unprecedented and simple example of the MCPBA-mediated cleavage of an aromatic double bond. The polyaromatic base system of **1** resembles a phenanthrenoid compound in terms of its chemical reactivity, and it is well known that the 9,10 double bond of phenanthrenic systems is labile and readily broken under oxidative conditions to yield di-aldehydes or acid derivatives.[30] Rings C/E of compound **1** (Scheme 1) would behave as a conjugated enol ether, which, after the expected epoxide formation and fragmentation, would lead to **2** that furnishes **3**, by further oxidation, and to the α-hydroxy-ketone **4**, by an acid-catalysed epoxide-acetal or α-hydroxy-hemiacetal hydrolysis (Scheme 2).

Although a more general view of the synthetic aspects of the described oxidation reaction will not be addressed in the present communication, the formation of the 10-membered lactone ring present in compounds **2** and **3** suggests a possible use of a phenanthrenic aromatic system linked face to face to an heterocyclic moiety in strategies for the synthesis of macrocyclic aromatic compounds. It is worth mentioning that medium-sized heterocycles (with 8 to 10-membered rings) are often found in organic chemistry as key intermediates in the synthesis of more complex structures or as core structures in natural products or pharmaceutically important compounds. Currently, the production of such rings remains an important challenge in organic synthesis.[31] Studies concerning the extended application of this methodology to other phenazinic systems are in progress.

Acknowledgements

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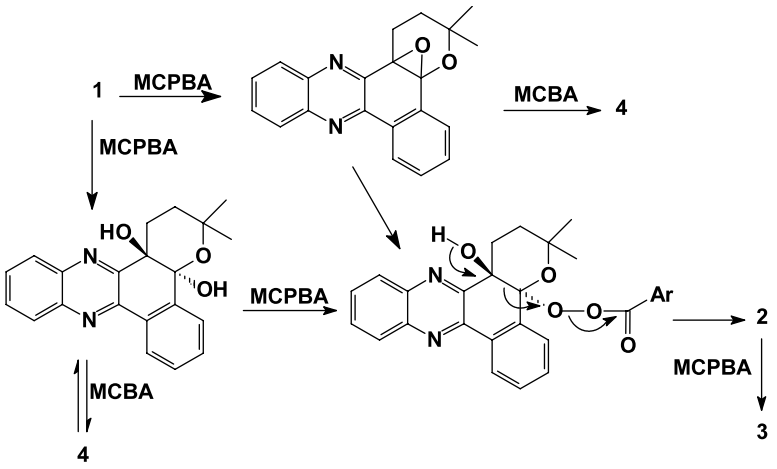

Scheme 2. The postulated oxidation mechanism for the formation of compounds **2**–**4** from phenazine **1**.

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15. Phenazine **1** (0.314 g, 1 mmol) in a solution of dichloromethane (7 mL) was treated at room temperature with *m*-chloroperbenzoic acid (MCPBA) (1.72 g, 10 mmol) added in small portions. The reaction was followed by TLC. After the usual work up, the reaction product residue was submitted to column chromatography over silica gel and eluted with mixtures of hexane/ethyl acetate of increasing polarity to furnish compounds **2**–**4**. Compound **2** was eluted with a mixture of hexane/ethyl acetate (100:1) and was recrystallized from acetone: the total yield of **2** in pure form was 27%. The two more polar products of the oxidation reaction, **3** and **4**, were isolated in yields of 13% and 35%, respectively.
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17. *7,7-dimethyl-7,8,9,10-tetrahydro-5H-benzo[3,4]oxecino-[5,6-b]quinoxaline-5,10-dione* (**2**) was obtained as colourless crystals, m.p. 162–163°C (corrected) after recrystallization from acetone. NMR experiments were performed with a solution of **2** in deuterochloroform containing TMS as the internal standard with a Bruker *AVANCE* DRX-400 instrument; chemical shifts are given on the δ-scale, and J-values are given in Hz. δ H [400 MHz, $CDCl_3$; J (Hz)] 1.36 (3H, s, CH_3); 1.66 (3H, s, CH_3); 1.98 (1H, ddd, $J=14.8$, 5.4 and 4); 2.28 (1H, ddd, $J=14.8$, 12.2 and 4), 2.75 (1H, ddd, $J=14.6$, 12.2 and 4); 3.81 (1H, ddd, $J=14.6$, 5.4 and 4); 7.57 (1H, td, $J=7.8$ and 1.6; ArH); 7.61 (1H, dd, $J=7.8$ and 1.6; ArH); 7.72 (1H, td, $J=7.6$ and 1.6; ArH); 7.83–7.87 (2H, m, 2ArH); 8.09 (1H, dd, $J=7.8$ and 1.6; ArH); 8.14–8.17 (2H, m, ArH). δ C (100 MHz, $CDCl_3$): 23.6 (CH_3); 27.0 (CH_3); 36.4 (CH_2); 38.5 (CH_2); 84.7 (C); 128.4 (C), 129.1 (CH); 129.2 (CH); 129.4 (CH); 130.6 (CH); 130.8 (CH); 131.2 (CH); 132.9 (CH); 133.0 (CH); 138.6 (C); 139.0 (C); 141.8 (C); 151.0 (C); 153.6 (C); 164.9 (CO_2); 201.9 (CO). IR KBr, cm^{-1}: 2960, 1720, 1700, 1600, 1300, 1140, 1060, 1040, 960, 760. λ_{max} (EtOH) nm (log ε): 327.8 (3.99); 245.0 (4.66); 208.4 (4.62). MS [70 ev, m/z (%)]: 346 (3); 331 (3); 318 (5); 303 (3); 287 (3); 262 (5); 249 (100); 233 (25); 220 (20); 204 (33); 177 (15); 102 (30); 83 (20); 76 (80); 50 (50). Found; C, 72.63%; H, 5.57%; N, 8.31%. Calc. for $C_{21}H_{18}N_2O_3$: C, 72.82%; H, 5.24%; N, 8.09%.
18. Crystal data for **2**: monoclinic, $P2_1/c$, $a=16.885(3)$, $b=9.792(2)$, $c=22.177(4)$ Å, $\beta=110.056(11)°$, $V=3444.34(11)$ Å^3, $Z=4$, $D_x=1.336$ mg m^{-3}, $\lambda(MoK_\alpha)=0.71073$ Å, $\mu=0.094$ mm^{-1}, $F(000)=1456$, $T=293$ K, 4582 unique observed reflections, $S=1.145$ and $R_1=0.053$.

19. *7,7-dimethyl-5,10-dioxo-7,8,9,10-tetrahydro-5H-benzo-[3,4]oxecino[5,6-b]quinoxaline-16-N-oxide* (**3**). White crystals, m.p. 212°C. δ H [400 MHz, $CDCl_3$; J (Hz)] 1.38 (3H, s, CH_3); 1.46 (3H, s, CH_3); 1.79 (1H, dt, $J_{gem}=14.8$, $J=5.4$ and 4); 2.32 (1H, td, $J_{gem}=14.6$, $J=12.2$ and 4), 2.73 (1H, dt, $J_{gem}=14.6$, $J=12.2$ and 4); 3.65 (1H, td, $J_{gem}=14.8$, $J=5.4$ and 4), 7.58 (1 H, d, ArH), 7.59 (1H, t, ArH), 7.72 (1H, t, ArH), 7.83 (1H, t, ArH), 7.89 (1H, t, ArH), 8.12 (1 H, d, ArH), 8.16 (1H, d, ArH), 8.61 (1H, d, ArH). δ C (100 MHz, $CDCl_3$): 22.8 (CH_3); 27.2 (CH_3); 36.8 (CH_2); 39.0 (CH_2); 84.4 (C); 119.7 (CH), 129.5 (C); 129.7 (CH); 130.1 (CH); 131.1 (CH); 131.4 (CH); 131.9 (CH); 132.2 (CH); 132.7 (CH); 137.6 (C); 139.2 (C); 142.2 (C); 153.4 (C); 164.4 (CO_2); 201.9 (CO). MS [70 ev, m/z (%)]: 362 (3); 346 (2); 330 (7); 302 (3); 287 (18); 265 (18); 249 (42); 233 (90); 221 (20); 204 (22); 177 (15); 130 (15); 102 (50); 83 (75); 76 (100). Found C, 68.85%; H, 5.13%; N, 7.66%. Calc. for $C_{21}H_{18}N_2O_4$ C, 69.60%; H, 5.01%; N 7.73%.
20. Monoclinic, $P2_1/c$, $a=12.206(2)$, $b=10.534(2)$, $c=14.685(4)$ Å, $\beta=110.63(5)°$, $V=1767.1(3)$ Å^3, $Z=4$, $D_x=1.370$ mg m^{-3}, $\lambda(MoK_\alpha)=0.71073$ Å, $\mu=0.099$ mm^{-1}, $F(000)=768$, $T=293$ K, 2744 unique observed reflections, $S=1.110$ and $R_1=0.057$.
21. Monoclinic, $P2_1/c$, $a=11.180(2)$, $b=19.697(6)$, $c=7.940(3)$ Å, $\beta=104.76(4)°$, $V=1690.7(4)$ Å^3, $Z=8$, $D_x=1.6$ mg m^{-3}, $\lambda(MoK_\alpha)=0.71073$ Å, $\mu=0.127$ mm^{-1}, $F(000)=832$, $T=293$ K, 2987 unique observed reflections, $S=1.082$ and $R_1=0.057$. The programmes employed were: cell determination and data collection—KappaCCD-Enraf-Nonius,(1999);[22] structure solution—SHELXS-86 (Sheldrick, 1997);[23] refinement—SHELXL-97 (Sheldrick, 1997);[23] graphic presentation—ORTEP3 (Farrugia, 1997);[24] software to prepare material for publication—WinGX (Farrugia, 1999).[25]
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26. *6-Hydroxy-6-(3-hydroxy-3-methylbutyl)-5,6-dihydrobenzo[a]phenazine-5-one* (**4**). White crystals, m.p. 206°C. IR (KBr), cm^{-1}: 3493, 3445, 3063, 2970, 2931, 2866, 2362, 2336, 1680, 1598, 1394, 1359, 1325, 1269, 1229, 1095, 943, 773. δ H [400 MHz, $CDCl_3$; J (Hz)]: 1.10 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.48 (1H, dq, $J=14.0$, 12.4, 4.7, H-1′), 1.64 (bs, OH), 1.65–1.57 (1H, m, H-2′), 2.11–1.97 (2H, m, H-1′), 4.63 (s, OH), 7.67 (1 H, dt, $J=7.5$, 1.2, ArH), 7.83–7.77 (2H, m, ArH), 7.85 (1H, dt, $J=7.5$, 1.2, ArH), 8.11 (1 H, dd, $J=7.5$, 1.2 Hz, ArH), 8.23–8.15 (2 H, m, ArH), 8.77 (1 H, d, $J=7.5$, ArH). δ C (100 MHz, $CDCl_3$): 29.3 (CH_3), 29.3 (CH_3), 36.7 (CH_2), 38.3 (CH_2), 70.3 (C), 81.1 (C), 125.9 (CH), 127.4 (CH), 129.2 (CH), 129.5 (CH), 130.5 (CH), 130.6 (C), 130.7 (CH), 131.6 (CH), 135.3 (CH), 135.8 (C), 141.2 (C), 142.3 (C), 143.8 (C), 153.7 (C), 199.3 (CO). MS [70 ev, m/z (%)]: 330 (3); 287 (3); 271 (3); 262 (3); 233 (26); 205 (2,7); 177 (11); 149 (51); 105 (10); 93 (7); 77 (19); 55 (14); 44 (100). Found C, 71.44%; H, 5.62%; N, 8.14%. Calc. for $C_{21}H_{20}N_2O_3$ C, 72.44%; H, 5.79%; N, 8.04%.
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