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Note

Preparation and NMR characterization of new substituted benzo[*a*]phenazines

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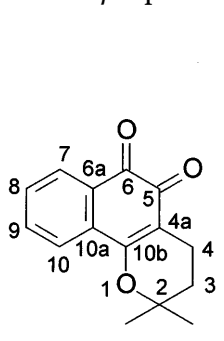
ABSTRACT: Benzo[*a*]phenazines were prepared by condensation of β-lapachone with 1,2-phenylenediamine and 4-chloro-1,2-phenylenediamine. The latter diamine gave two regioisomers that could be separated and unambiguously identified by means of their ^{1}H and ^{13}C NMR spectra with the aid of 2D NMR experiments, mainly HETCOR and COLOC. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; β-lapachone; benzo[*a*]phenazines

INTRODUCTION

Benzo[*a*]phenazine derivatives are efficient DNA intercalating ligands which have shown to possess antitumor activity when evaluated *in vitro* and *in vivo* against leukemia and solid tumor models.[1] On the other hand, β-lapachone (2,2-dimethyl-3,4,5,6-tetrahydro-2*H*-oxine-5,6-dione, **1**) is a naturally occurring *o*-naphthoquinone with antineoplastic activity.[2] These structural features may be combined to give benzo[*a*]phenazine analogs of β-lapachone (**1**) by condensation of the *o*-quinone with 1,2-phenylenediamines. We report here the synthesis and NMR spectral assignment of derivatives **2**, **3** and **4**. Regiochemistry of the chlorine-substituted isomers **2** and **3** could be unambiguously established by means of 2D NMR spectroscopy, mostly COLOC and HETCOR. The complete assignment of the ^{13}C NMR spectrum of β-lapachone is also given.



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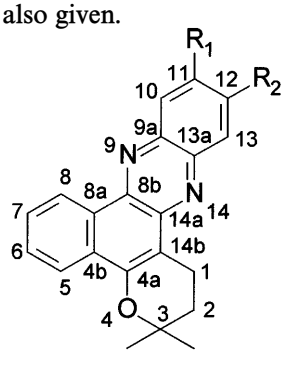

2 R_1 = Cl, R_2 = H
3 R_1 = H, R_2 = Cl
4 R_1 = R_2 = H

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RESULTS AND DISCUSSION

Chemistry

Reaction of β-lapachone with phenylenediamines in refluxing methanol gave the corresponding bright yellow benzo[*a*]phenazines. When 4-chloro-1,2-phenylenediamine was used, the two possible isomers were obtained. The preparation of other benzo[*a*]phenazines monosubstituted at C-11 or C-12 has been reported[3,4] but no assignment of the NMR spectra has been made. The new compounds were characterized by ^{1}H and ^{13}C NMR and mass spectrometry.

β-Lapachone (1)

The complete assignment of the benzo[*a*]phenazine spectra required the unambiguous assignment of the ^{1}H and ^{13}C NMR spectra of β-lapachone. Table 1 shows the spectral data and assignments, which differ from those reported previously.[5] The aromatic hydrogen resonances were assigned based on their multiplicities and by comparison with the spectrum of 8,9-dimethoxy-β-lapachone described by Shaffner-Sabba *et al.*[6] In this compound, H-7 next to the carbonyl resonates downfield compared with H-10, next to the ether group. Hence the double doublets at 8.06 and 7.82 ppm were assigned to H-7 and H-10, respectively. Selective decoupling of these hydrogens allowed us to distinguish between H-8 and H-9. Assignment of H-3 and H-4 was straightforward based on their chemical shifts. These assignments were corroborated by means of H–C long-range correlations from the COLOC experiment (see below). Methyne and methylene ^{13}C NMR signals were then assigned by their one-bond H–C correlations in a

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Table 1. ^{1}H and ^{13}C NMR spectral data [δ (ppm) and J (Hz)] for β-lapachone (**1**) in deuteriochloroform

	H	C	H–C long-range correlations[a]
2	—	79.1	H-4 (^3J), CH_3 (^2J)
3	1.86 (t, J = 6.6)	31.4	CH_3 (^3J)
4	2.58 (t, J = 6.6)	16.0	H-3 (^2J)
4a	—	112.5	H-3 (^3J), H-4 (^2J)
5	—	178.3	H-4 (^3J)
6	—	179.6	H-7 (^3J)
6a	—	130.0[b]	H-10 (^3J), H-8 (^3J)
7	8.06 (dd, J = 1.4, 7.5)	128.3	H-8 (^2J)
8	7.50 (dt, J = 1.2, 7.4)	130.5	H-10 (^3J)
9	7.64 (dt, J = 1.4, 7.6)	134.6[c]	H-7 (^3J)
10	7.82 (dd, J = 1.0, 7.7)	123.9	H-8 (^3J)
10a	—	132.4[b]	H-9 (^3J), H-7 (^3J)
10b	—	161.8	H-10 (^3J), H-4 (^3J)
CH_3	1.47 (s)	26.6	

[a] From COLOC experiment.
[b,c] Pairs of assignments corrected from Ref. 5.

HETCOR experiment. Carbonyl C-5 was distinguished from C-6 by the long-range correlations with H-4 and H-7, respectively, observed in the COLOC experiment. The chemical shift of C-10b (161.8 ppm) is indicative of a quaternary sp^2 carbon bonded to an oxygen. The expected long-range correlations with H-4 and H-10 were also observed; the latter confirmed the assignment of the aromatic methynes described above. C-4a was easily assigned by its sp^2 chemical shift (112.5 ppm) and the long-range correlation with H-3. To distinguish C-6a from C-10a, the long-range correlations with the pairs H-8/H-10 and H-7/H-9, respectively, were conclusive.

Chlorine-substituted benzo[*a*]phenazines 2 and 3

Prior to the assignment of the NMR spectra of **2** and **3**, both regioisomers had to be unambiguously identified. In these compounds the chlorinated aromatic ring is isolated from the rest of the molecule by the heterocyclic pyrazine, hence no long range H–C correlations

Table 2. ^{1}H and ^{13}C NMR spectral data [δ (ppm) and J (Hz)] for benzophenazines **2** and **3** in deuteriochloroform

	2			**3**		
	H	C	H–C long-range correlations[a]	H	C	H–C long-range correlations[a]
1	3.29 (t, J = 6.6)	18.1	—	3.30 (t, J = 6.7)	18.1	—
2	2.07 (t, J = 6.6)	32.3	CH_3 (^3J)	2.07 (t, J = 6.7)	32.3	CH_3 (^3J)
3	—	76.1	CH_3 (^2J), H-1 (^3J)	—	76.1	H-1 (^3J), H-2 (^2J), CH_3 (^2J)
4a	—	152.2	H-1 (^3J), H-5 (^3J)	—	151.7	H-5 (^3J), H-1 (^3J)
4b	—	129.2	H-8 (^3J)	—	129.3	H-8 (^3J)
5	8.31 (m)	122.1	—	8.32 (m)	122.0	—
6	7.77 (m)	129.5	—	7.78 (m)	129.7	H-8 (^3J), H-5 (^2J)
7	7.75 (m)	127.6	—	7.75 (m)	127.6	H-5 (^3J)
8	9.29 (m)	124.9	—	9.29 (m)	125.1	—
8a	—	130.3	H-7 (^3J), H-5 (^3J)	—	130.2	H-7 (^3J), H-5 (^3J)
8b	—	139.9	H-8 (^3J)	—	140.3	H-8 (^3J)
9a	—	142.5	H-13 (^3J)	—	140.8	H-13 (^3J), H-11 (^3J)
10	8.23 (d, J = 2.3)	127.3	—	8.14 (d, J = 9)	129.8	—
11	—	135.0	H-13 (^3J)	7.72 (dd, J = 2.3, 9)	130.2	H-13 (^3J)
12	7.68 (dd, J = 2.3, 9)	128.8	—	—	133.3	H-13 (^2J), H-10 (^3J)
13	8.22 (d, J = 9)	130.6	—	8.29 (d, J = 2.3)	128.0	—
13a	—	138.4	H-12 (^3J), H-10 (^3J)	—	140.0	H-10 (^3J)
14a	—	144.8	H-1 (^3J)	—	144.3	H-1 (^3J)
14b	—	109.3	H-1 (^2J), H-2 (^3J)	—	109.4	H-1 (^2J), H-2 (^3J)
CH_3	1.53 (s)	26.8	—	1.53 (s)	26.7	—

[a] From COLOC experiment.

Table 3. ^{1}H and ^{13}C NMR spectral data [δ (ppm) and J (Hz)] for benzophenazine **4** in deuteriochloroform

	H	C	H–C long-range correlations[a]
1	3.34 (t, J = 6.5)	18.4	—
2	2.07 (t, J = 6.5)	32.5	CH_3 (^3J)
3	—	76.1	H-2 (^2J), H-1 (^3J), CH_3 (^2J)
4a	—	151.6	H-5 (^3J)
4b	—	129.4	H-6 (^3J)
5	8.33 (m)	122.2	H-7 (^3J)
6	7.76 (m)	129.5	H-8 (^3J)
7	7.78 (m)	127.6	—
8	9.34 (m)	125.1	H-6 (^3J)
8a	—	130.6	H-7 (^3J), H-5 (^3J)
8b	—	140.2	H-8 (^3J)
9a	—	142.6	H-11 (^3J), H-13 (^3J)
10	8.24 (m)	128.8	H-12 (^3J)
11	7.78 (m)	129.4	H-13 (^3J)
12	7.78 (m)	128.0	H-10 (^3J)
13	8.30 (m)	129.6	H-11 (^3J)
13a	—	140.2	H-10 (^3J), H-12 (^3J)
14a	—	144.3	H-1 (^3J)
14b	—	109.7	H-2 (^3J), H-1 (^2J)
CH_3	1.53 (s)	26.9	—

[a] From COLOC experiment.

are available to correlate those ring nuclei (H or C) to known β-lapachone related signals. Furthermore, molecular modeling calculations (AM1, AMPAC 5.01) predicted a distance of >4 Å between H-8 and H-10, thus precluding the use of a NOESY experiment to assign the latter hydrogen.

In benzo[*a*]phenazine systems it has been established that the hydrogen inside the concave side of the molecule, H-8, is downfield compared with H-5 on the convex side. On the other hand, on the benzene ring next to the heterocycle, H-10 is upfield compared with H-13 on the convex side.[7] These differences were used to distinguish between the regioisomers. The isolated hydrogen in both isomers (*i.e* H-10 in **2** and H-13 in **3**) can be easily identified from its multiplicity and small coupling constant (Table 2). Further, the regioisomer with this H at higher field must be **2** (H-10 at 8.23 ppm) and the other one **3** (H-13 at 8.29 ppm). Concomitantly, H-13 in **2** resonated at lower field than H-10 in **3** (8.22 *vs.* 8.14 ppm). This is in agreement with the substitution pattern of each regioisomer.

The other ^{1}H NMR signals of these compounds could be identified by comparison with β-lapachone. ^{13}C NMR signals were assigned from the H–C correlations obtained from HETCOR (one-bond) and COLOC (two- and three-bond) experiments; all data were in agreement with the assignments shown in Table 2.

Benzo[*a*]phenazine 4

The assignment of the ^{1}H NMR spectrum of this compound was complicated by the fact that the signals of H-6, H-7, H-11 and H-12 are heavily overlapped, rendering an unresolved multiplet at 7.75 ppm (Table 3). Among the signals at *ca.* 8.3 ppm, H-5 was assigned to the resonance at 8.33 ppm by H–C correlation and comparison with **2** and **3**. H-13 and H-10 were at 8.30 and 8.24 ppm; in this case, again we considered that H-10 in the concave side is shielded compared with H-13. The latter assignment was confirmed by long-range H–C correlations in the COLOC experiment.

The ^{13}C NMR spectrum of **4** showed only 18 signals for the 20 carbons (Table 3). A quaternary carbon could be found by a QUAT experiment at 129.4 ppm. From long-range H–C correlations it could be found that C-11 and C-4b overlapped at this chemical shift value (Fig. 1) and that the signal at 142.6 ppm corresponded to the resonance of C-9a. Signals at 144.3 and 140.2 ppm were assigned to C-14a and C-8b, respectively, by comparison with **2** and **3** and confirmed by the observed long-range H–C correlations. Besides the correlation for C-8b (H-8), the signal at 140.2 ppm showed cross peaks at 8.24 ppm (H-10) and at 7.78 ppm (H-12). These correspond to the expected three-bond correlations for C-13a, which must then overlap with C-8b.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were measured at 200.13 and 50.32 MHz, respectively, in a Bruker AC-200 NMR spectrometer in deuteriochloroform (with tetramethylsilane as internal standard) using standard Bruker software. All spectra were recorded at 303 K. Typical conditions for ^{13}C NMR spectra were 20 000

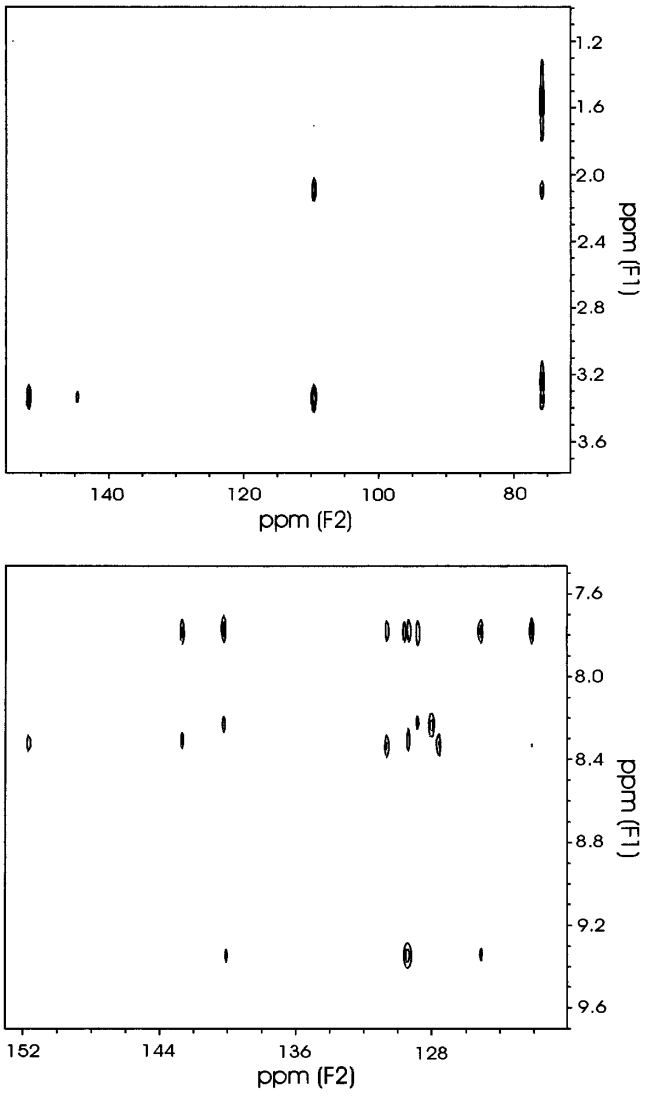

Figure 1. Expansions of the COLOC spectrum of **4** showing C–H correlations through $^{2}J_{CH}$ and $^{3}J_{CH}$.

transients, 16K data points, 4.4 μs pulses (45° flip angle), 11 000 Hz spectral width, 0.74 s acquisition time and no relaxation delay. For ^{1}H NMR spectra a 3000 Hz spectral width was used and a 2.2 s acquisition time with a 16K data table.

^{1}H–^{13}C shift correlation spectra (XHCORRD in the Bruker operating software) were measured in the absolute value mode with ^{1}H–^{1}H decoupling in F_1 with the standard Bruker sequence. A total of 256 data points for the t_1 dimension (1000 Hz spectral width) and 2048 data points for the t_2 dimension (5500 Hz spectral width) were used. The FIDs were Fourier transformed on a 1K × 2K data matrix using shifted squared sine-bell window functions in both dimensions.

Long-range heteronuclear 2D ^{1}H–^{13}C shift correlation spectra were obtained using the standard sequence (COLOC in the Bruker operating software). The spectra were acquired with 4K × 256 data points and a data acquisition of 128 scans × 128 increments in the t_1 dimension. Spectral widths of 700–900 Hz for F_1 and 6500–7500 Hz for F_2 were used. The relaxation delay was 1 s, the refocusing delay was 15–25 ms and the corresponding polarization delay was twice this value. Data were processed using shifted squared sine-bell functions in both dimensions.

Melting points were determined with a Fisher–Johns apparatus and are uncorrected. β-Lapachone (m.p. 155–156 °C) was isolated from heartwood of *Tabebuia avellanedae*[8] and also prepared by acid cyclization of lapachol[9] isolated from the same source. 4-Chloro-*o*-phenylenediamine was obtained by reduction of the 4-chloro-2-nitroaniline with Zn in 20% NaOH ethanolic solution.[10]

11-Chloro-3,3-dimethyl-2,3-dihydro-1*H*-benzo[*a*]oxino[2,3-*c*]phenazine (2) and 12-chloro-3,3-dimethyl-2,3-dihydro-1*H*-benzo[*a*]oxino[2,3-*c*]phenazine (3). β-Lapachone (0.3 g) and 4-chloro-*o*-phenylenediamine (0.21 g) were dissolved in methanol (10 ml). The mixture was stirred under reflux for 3 h, giving a yellow precipitate. Evaporation of the solvent followed by flash chromatography of the residue eluting with toluene–hexane (4:5) afforded **2** (120 mg, 42%), m.p. 161–162 °C, EIMS *m/z* 348 (M^+, 40), 333 (8), 305 (100), 293 (42), and **3** (80 mg, 28%), m.p. 168.5–169 °C, EIMS *m/z* 348 (M^+, 21), 333 (4), 305 (51), 293 (23), 41 (100).

3,3-Dimethyl-2,3-dihydro-1*H*-benzo[*a*]oxino[2,3-*c*]phenazine (4). β-Lapachone (1.00 g) and *o*-phenylenediamine (0.55 g) were dissolved in methanol (15 ml). The mixture was stirred under reflux for 3 h, giving a yellow precipitate. Evaporation of the solvent followed by flash chromatography of the residue eluting with hexane–ethyl acetate (8:2) afforded **4** (1.27 g, 98%), m.p. 133.5 °C (lit.[11] 130.5–133.5 °C), EIMS *m/z* 314 (M^+, 44), 299 (7), 285 (7), 271 (100).

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REFERENCES

1. G. W. Rewcastle, W. A. Denny and B. C. Baguley, *J. Med. Chem.* **30**, 843 (1987).
2. (a) D. A. Boothman and A. B. Pardee, *Proc. Natl. Acad. Sci. USA*, **86**, 4963 (1989); (b) S. M. Planchon, S. Wuerzberger, B. Frydman, D. T. Witiak, P. Hutson, D. R. Church, G. Wilding and D. A. Boothman, *Cancer Res.* **55**, 3706 (1995); (c) C. J. Li, C. Wang and A. B. Pardee, *Cancer Res.* **55**, 3712 (1995).
3. H. J. Kallmayer, *Pharm. Acta. Helv.* **64**, 242 (1989).
4. N. Watanabe, I. Mitsumoto, K. Komiyama and R. Shimazu, *Tokyo Kogyo Koto Senmon Gakko Kenkyu Hokokusho* **22**, 99 (1990).
5. I. A. McDonald, T. J. Simpson and A. F. Sierakowski, *Aust. J. Chem.* **30**, 1727 (1977).
6. K. Shaffner-Sabba, K. H. Schmidt-Ruppin, W. Wehrli, A. R. Schuerch and J. W. F. Wasley, *J. Med. Chem.* **27**, 991 (1984).
7. H. J. Kallmayer, *Pharm. Acta Helv.* **63**, 242 (1988).
8. O. Gonçalves de Lima, I. L. D'Aalbuquerque, G. M. Maciel and M. C. N. Maciel, *Rev. Inst. Antibiot. (Recife)* **8**, 95 (1968).
9. S. C. Hooker, *J. Chem. Soc.* **61**, 611 (1892).
10. E. L. Martin, *Org. Synth. Coll. Vol.* **2**, 501 (1943).
11. S. C. Hooker, *J. Am. Chem. Soc.* **58**, 1190 (1936).