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Reference Data

Complete ^{13}C and ^{1}H Spectral Assignments of Certain Substituted Quinoxalinones

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ABSTRACT: Two tagging reagents using a quinoxalinone fluorophore were synthesized and, for the first time, completely physico-chemically characterized. The ^{1}H and ^{13}C spectra were entirely assigned using a combination of two-dimensional gradient enhanced HMQC, HMBC experiments and one-dimensional NOE difference experiments. © 1997 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; quinoxalinones

INTRODUCTION

For two decades, several groups have been investigating and reporting the use of quinoxalinone fluorophores in the high-performance liquid chromatographic trace determination of analyte-bearing functional groups.[1–8] Of the quinoxalinone fluorophores, it has been shown that the 6,7-dimethoxy-1-methyl-2-(1*H*)-quinoxalinone (DMEQ) moiety gives the highest fluorescent signal.[9] Five different tagging reagents using this moiety and a specially adapted functional lateral chain were developed successively (Br-DMEQ, DMEQ-COCl, DMEQ-CON_3, DMEQ-hydrazide and DMEQ-TAD). Two of them, Br-DMEQ[2] and DMEQ-hydrazide,[7] specifically developed for the carboxylic acid groups as a derivatization site, were employed in a metabolism study. These two labelling reagents, not commercially available, were therefore synthesized and physico-chemically characterized. Dave *et al.*[6] previously assigned the *N*- and *O*-methylated structures occurring during the methylation step of the DMEQ-COCl synthesis by using a one-dimensional NOE difference experiment (see Scheme 1). The complete NMR ^{1}H and ^{13}C assignment of each synthon of the two syntheses (Br-DMEQ and DMEQ-hydrazide) has not previously been published. Using a combination of two-dimensional gradient enhanced HMQC, HMBC experiments and one-dimensional NOE difference experiments, these assignments have now been fully achieved. An unexpectedly[2] poor chemical stability of Br-DMEQ prevented us from achieving the same protocol on this structure.

EXPERIMENTAL

Chemicals purchased from Aldrich and Fluka were of the highest available quality and were used without further purification. Melting points were left uncorrected. Infrared spectra were obtained using a Nicolet Model 710 FT-IR instrument. Electron impact mass spectra were recorded with a Nermag R10-10 C quadrupolar instrument.

DQ, 6,7-dimethoxy-3-methyl-2(1*H*)-quinoxalinone (1)

This was synthesized according to the literature procedure;[9] m.p. 255 °C; IR (in KBr), $\nu = 3422$ (NH), 3073 (aromatic), 1664 (C=O amide), 1507 (aromatic C—C), 1258 (C—O—C) cm^{-1}; MS, *m/z* 220 (M^+, 100%), 205 (24), 177 (12), 149 (14).

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DDQ, 6,7-dimethoxy-1,3-dimethyl-2(1*H*)-quinoxalinone (1a)

Compound **1a** was synthesized according to a modified[10] described procedure;[2] m.p. 170 °C; IR (in KBr), $\nu = 2959$ (C—H), 1644 (C=O amide), 1511 (aromatic C–C), 1242 (C—O—C) cm^{-1}; MS, *m/z* 234 (M^+, 100%), 219 (39), 191 (43), 163 (25).

Br-DMEQ, 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone (1b)

Compound **1b** was synthesized according to a modified[10] described procedure;[2] m.p. 161 °C; IR (in KBr), $\nu = 3042$ (aromatic), 1649 (C=O amide), 1510 (aromatic C–C) cm^{-1}; MS, *m/z* 314 ($M^+ + 2$, 11%), 312 (M^+, 11), 234 (100), 205 (11), 161 (11).

DQP, 6,7-dimethoxy-2(1*H*)-quinoxalinone-3-propionylcarboxylic acid (2)

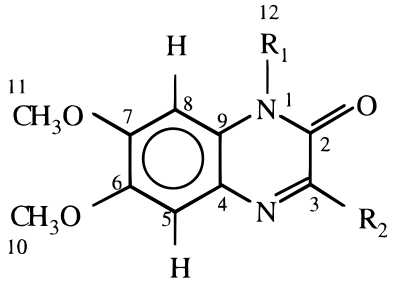
Compound **2** was synthesized according to the procedure described in the literature;[9] m.p. 245 °C; IR (in KBr), $\nu = 3063$ (aromatic), 3350–2670 (CO_2H), 1714 (C=O acid), 1642 (C=O amide), 1503 (aromatic C—C), 1257 (C—O—C) cm^{-1}; MS, *m/z* 278 (M^+, 61%), 260 (27), 247 (29), 233 (87), 219 (27), 205 (17).

DMQP, 6,7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone-3-propionylcarboxylic acid methyl ester (2a)

Compound **2a** was synthesized from DQP according to a modified[10] described procedure;[11] m.p. 176 °C; IR (in KBr), $\nu = 1738$ (C=O ester), 1647 (C=O amide), 1620 (aromatic C=N), 1514 (aromatic C—C), 1271 (C—O—C) cm^{-1}; MS *m/z* 306 (M^+, 84%), 274 (34), 247 (100), 233 (34), 203 (31), 175 (12).

DMEQ-hydrazide, 6,7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone-3-propionylcarboxylic acid hydrazide (2b)

Compound **2b** was synthesized according to a modified[10] described procedure;[7] m.p. 205 °C; IR (in KBr), $\nu = 3296$ (NH), 3036 (aromatic), 2937 (C—H), 1643 (C=O amide, hydrazide), 1514 (aromatic C—C), 1246 (C—O—C) cm^{-1}; MS, *m/z* 306 (M^+, 34%), 275 (67), 247 (53), 233 (100), 203 (14).



1 R_1=H R_2=$^{13}CH_3$

1a R_1=CH_3 R_2=$^{13}CH_3$

2 R_1=H R_2=$^{14}CH_2{}^{15}CH_2{}^{16}COO{}^{17}H$

2a R_1=CH_3 R_2=$^{14}CH_2{}^{15}CH_2{}^{16}COO{}^{17}CH_3$

2b R_1=CH_3 R_2=$^{14}CH_2{}^{15}CH_2{}^{16}CO{}^{17}NH{}^{18}NH_2$

CCC 0749–1581/98/040300–03 $17.50

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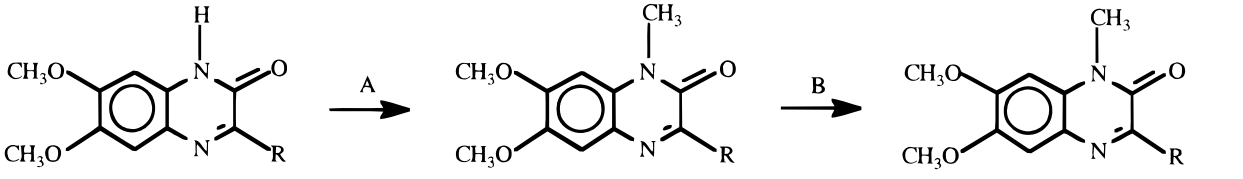

1 R=Me
2 R=CH_2CH_2COOH

1a R=Me
2a R=CH_2CH_2COOMe

1b R=CH_2Br
2b R=$CH_2CH_2CONHNH_2$

A : methylation B : bromination or hydrazination

Scheme 1

Table 1. ^{1}H NMR assignments for compounds 1–2b[a]

Position	1[b]	1a[c]	2[c]	2a[c]	2b[c]
5	7.18 (s, 1H)	7.18 (s, 1H)	7.15 (s, 1H)	7.14 (s, 1H)	7.22 (s, 1H)
8	6.74 (s, 1H)	6.88 (s, 1H)	6.76 (s, 1H)	6.93 (s, 1H)	6.95 (s, 1H)
10	3.79 (s, 3H)	3.83 (s, 3H)	3.81 (s, 3H)	3.84 (s, 3H)	3.83 (s, 3H)
11	3.81 (s, 3H)	3.94 (s, 3H)	3.81 (s, 3H)	3.93 (s, 3H)	3.93 (s, 3H)
12	12.13 (s, 1H)	3.60 (s, 1H)	12.12 (s, 1H)	3.64 (s, 3H)	3.63 (s, 3H)
13	2.34 (s, 3H)	2.38 (s, 3H)	—	—	—
14	—	—	2.95 (t, 2H, 6.9[d])	3.04 (t, 2H, 7.1)	2.99 (t, 2H, 7.6)
15	—	—	2.68 (t, 2H, 6.9)	2.79 (t, 2H, 7.1)	2.50 (t, 2H, 7.6)
16	—	—	—	—	9.01 (s, 1H)
17	—	—	12.12 (s, 1H)	3.61 (s, 3H)	4.16 (s, 2H)

[a] δ (ppm) relative to solvent DMSO. Assignments are based on the NOE experiments.
[b] 400.13 MHz.
[c] 250.13 MHz.
[d] Coupling constants in Hz.

NMR spectroscopy

2D NMR data were recorded at 9.4 T on a Bruker Avance DRX spectrometer equipped with a multinuclear inverse 5 mm probe with a one-axis gradient coil operating at 298 K (^{1}H, 400.13 MHz, 90° pulse width = 7.8 μs; ^{13}C, 100.62 MHz, 90° pulse width = 14.5 μs). Samples contained 60–80 mg of a specific compound dissolved in dimethyl-d_6 sulphoxide with a trace of tetramethylsilane added as an internal ^{1}H and ^{13}C chemical shift reference. ^{13}C NMR spectra were recorded using a spectral width of 20 000 Hz with 32K complex points and zero filling of 32K. Gradient enhanced HMQC[12–14] experiments were carried out using the TPPI procedure to obtain pure absorptive signals. Gradient amplitudes were 25/15/20 G cm^{-1} in the HMQC and HMBC experiments and GARP decoupling was used on the ^{13}C for the HMQC experiment. The results of the gradient enhanced HMBC experiments[14–16] were invaluable in assigning aromatic resonances; a typical value of 60 ms was used for the evolution of long-range coupling, and a value of 3 ms was used for the low-pass J filter. For both the HMQC and HMBC experiments, the acquisition parameters were as follows: ^{1}H spectral width of 4000 Hz; ^{13}C spectral width of 20 000 Hz; 2K points in the ^{1}H dimension; 1K increments in the ^{13}C dimension (this large number of points in the ^{13}C dimension was essential to ascribe the C_{10} and C_{11} chemical shifts in the final 2D map); a 1.5 s relaxation delay; and 16–32 transients per increment. HMQC and HMBC spectra were processed with a Gaussian window function in both dimensions. 1D NOE difference[17,18] spectra were recorded at both 9.4 and 5.87 T; 16–32 transients of 8K points for reference and irradiated spectra were performed with a 6 s presaturation time. Experiments were repeated eight times consecutively and then added together to minimize artefacts due to instrument instabilities. Samples were not degassed for the NOE measurement. The target irradiation protons were the two aromatic protons which were easily identifiable in the spectrum of each compound.

Table 2. ^{13}C NMR assignments for compounds 1–2b[a]

Carbon	1	1a	2	2a	2b
2	154.80	155.66	154.52	153.98	153.91
3	155.29	155.65	156.51	154.67	155.39
4	125.89	127.48	125.72	126.01	125.93
5	109.09	110.93	109.28	110.48	110.07
6	145.53	146.52	145.61	145.68	145.44
7	150.63	151.51	150.75	151.07	150.75
8	96.93	96.43	96.93	97.39	97.11
9	126.64	128.95	126.45	127.88	127.73
10	55.90	56.72	55.74	55.92	55.72
11	55.72	56.84	55.74	56.18	56.06
12	—	29.78	—	29.13	29.13
13	20.19	21.90	—	—	—
14	—	—	27.51	28.11	29.01
15	—	—	29.75	29.64	29.77
16	—	—	174.04	172.85	171.08
17	—	—	—	51.23	—

[a] δ(ppm) relative to solvent DMSO, 100.62 MHz. Assignments are based on the NOE, HMBC and HMQC experiments.

RESULTS AND DISCUSSION

^{1}H and ^{13}C NMR data are given in Tables 1 and 2, respectively.

The initial assignments obtained in this work were achieved by means of proton–carbon correlation methods, specifically the HMQC experiment for direct correlation, and HMBC experiment for long-

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range coupling. It is the symmetry of the aromatic part of the quinoxalinone moiety which proved to be most awkward when attempting to assign all the chemical shifts. HMBC experiments allow the attribution of the ^{1}H–^{13}C coupling network without discrimination. A nuclear Overhauser enhancement of proton(s) in the R_1 group was observed at the same time as an NOE of protons involved in one of the methoxy groups, indicating that the target proton was H_8. For each compound, irradiation of the second aromatic proton gave an NOE of protons involved in the other methoxy group, which confirmed the previous assignment. The 1D NOE difference experiments were essential to assign the ^{1}H and ^{13}C spectra completely. It was noticed that the chemical shifts of C_{10} and C_{11} were close and that their values were strongly dependent on the R_1 and R_2 substitution groups.

CONCLUSION

The proton and carbon chemical shifts and the assignment of certain substituted quinoxalinones were determined using the HMQC, HMBC and NOE difference experiments. These data appear to represent the first complete assignment of this family of molecules, and will be useful in future work in terms of structural elucidation.

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