SYNTHESIS OF DEUTERIUM LABELLED ANALOGS OF THE UBIQUINONE HEADGROUP

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Summary

Deuterium labelled analogs of 2,3 – dimethoxy – 5 – methyl – 1,4 – benzoquinone (2,3 – dimethoxy – 5 – methyl[$^2H_3$] – 1,4 – benzoquinone; 2,3 – dimethoxy[$^2H_6$] – 5 – methyl – 1,4 – benzoquinone; 2,3 – dimethoxy[$^2H_6$] – 5 – methyl[$^2H_3$] – 1,4 – benzoquinone) were prepared in five steps starting from 3,4,5 – trihydroxybenzoic acid. The proposed synthetic route results in 100% specific labelling and gives good overall yields. Mass spectra of the labelled derivatives will be briefly discussed.

Key words: 2,3 – Dimethoxy – 5 – methyl – 1,4 – benzoquinone, Ubiquinone, deuterium, mitochondrial respiratory chain.

Introduction

The structure of the respiratory chain component ubiquinone (1) (coenzyme Q_{10}; see Fig. 1) has been known for a long time (1). The details of its function in electron transport and proton translocation (2,3) in mitochondria and in photosynthetic systems are however still under debate. A fundamental understanding of these processes can be expected only from biophysical studies on the interaction of this molecule with model and natural membranes.

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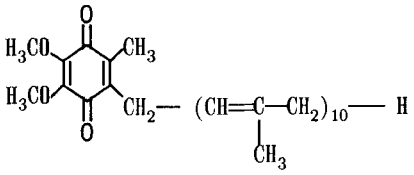


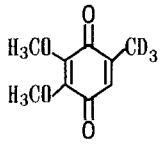
Figure 1: Ubiquinone (Coenzyme Q_{10}), (1)

The interaction of hydrophobic molecules with biological membranes can be studied most elegantly by deuterium nuclear magnetic resonance. Thus, the ubiquinone headgroup (2,3 – dimethoxy – 5 – methyl – 1,4 – benzoquinone (2)) was selectively labelled with deuterium leading to a precursor for the synthesis of labelled ubiquinones with saturated alkyl– or isoprenoid side chains (4 – 9).

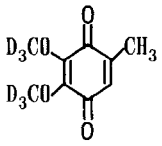
In a recent study deuterium was introduced biosynthetically into the coenzyme molecule (10) although without specific incorporation. Also the exchange of the methoxy groups for methoxy–[$^2H_3$] groups has been described (4). However, this procedure unfortunately gives a very low yield (10%; 47% exchange) and the chemical exchange proceeds under rather drastic reaction conditions.

The chemical synthesis of the selectively deuterated ubiquinone headgroup followed by the attachment of an appropriate side chain using published procedures (4 – 9) avoids these difficulties. The synthesis of (2) has been described previously starting from 3 – methoxy – 4 – hydroxybenzaldehyde (11 – 16), 3,4,5 – trimethoxybenzoic acid (16 – 20), 2,3,4 – trimethoxybenzaldehyde (18, 21) or 2,3 – dimethoxyphenol (22).

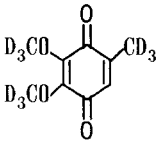
We choose 3,4,5 – trihydroxybenzoic acid as the starting compound since deuterated derivatives of the other precursors are difficult to obtain. The key step in the synthesis of the deuterium labelled analogs (2a), (2b), and (2c) (cf. Fig. 2) is a reductive methylation with sodium borohydride (18, 29) (cf. scheme 1). This published procedure has been modified appropriately.



(2a)



(2b)



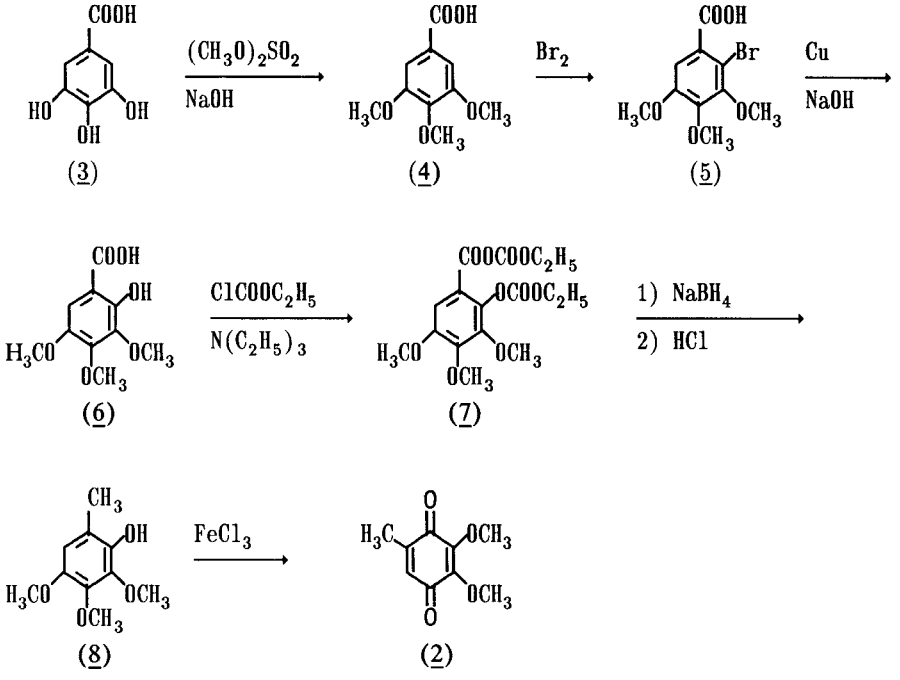
(2c)

Figure 2

Results and Discussion

Synthesis of 2,3 - dimethoxy - 5 - methyl[$^2H_3$] *1,4 – benzoquinone (2a)*

The preparation of (2a) by reductive methylation (18) as outlined in scheme 1 starts from 3,4,5 – trimethoxybenzoic acid (4) which is easily obtained by alkylation of 3,4,5 – trihydroxybenzoic acid (3) with dimethylsulfate (23). Attempts to prepare (6) by bromination of 3,4,5 – trimethoxybenzoic acid and exchange of the bromine atom in the presence of copper powder in 3 M NaOH (24) led to unsatisfactory results. Dark by–products which had probably formed due to the long reaction times (50 hr for (5) and 7 hr for (6)) were difficult to remove. Thus, (5) and (6) were obtained in rather low yield. Also reduction during the synthesis of (6) leading to noticeable quantities of (4) was observed. Moreover, purification of the final product on silica gel was not possible due to the decomposition of (6).



Scheme 1

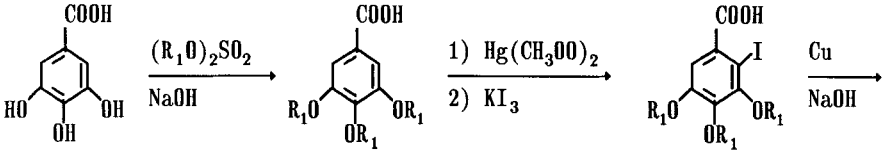
Instead of employing the brominated analog as the intermediate the synthesis of (6) was achieved by using 2 – iodo – 3,4,5 – trimethoxybenzoic acid ((9), cf. scheme 2).

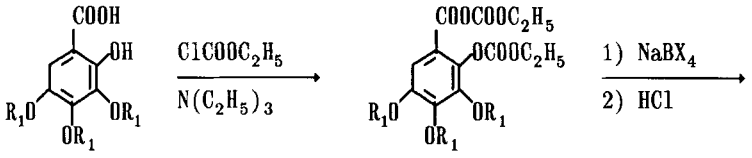
This proved advantageous since the reaction goes to completion within one hour under the same conditions as described for the brominated compound (24). The total procedure is summarized in scheme 2.

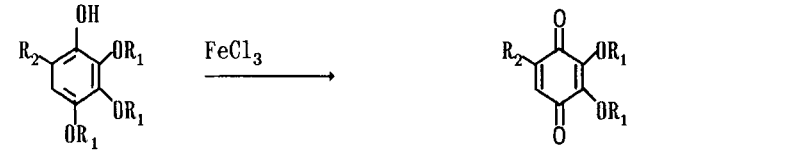
The preparation of (9) has been described in ref. 24 starting from (5). However this route involves the problems mentioned above. As an alternative, (4) can be treated with mercuric (II) acetate and sodium chloride. The chloromercuric compound thus obtained yields (9) after reaction with iodine in ethanol (25). This represents a customary procedure for the introduction of iodine into aromatic rings (26 – 28).

The iodination of (4) was performed in one step since we found it unnecessary to isolate the chloromercuric compound. Thus (4) was heated with mercuric (II) acetate and with a catalytic amount of acetic acid in water. Addition of an aqueous solution of potassium iodide and iodine and further heating gave the desired product (9) with good yield (77.1%) and with excellent purity. After the subsequent synthesis of (6) only negligible formation (about 4.5%) of (4) was observed by ^1H NMR. The complete removal of (4) was only achieved after repeated recrystallization from water or from a cyclohexane/toluene mixture. The raw product could be used without wasteful recrystallization, however, since the presence of (4) does not interfere with the subsequent synthetic steps.

The final product (2a) was prepared basically following published procedures (18). Reaction of (6) with ethoxycarbonylchloride and triethylamine yielded the bis – (ethoxycarbonyl) – compound (7). Reduction of (7) was performed by sodium borohydride – $[^2H_4]$. The desired quinone was obtained by oxidation of (8a) by iron (III) chloride (18; cf. scheme 2). Purification of the final product was conveniently achieved by sublimation of the crystalline material.







X = D; $R_1 = CH_3$; $R_2 = CD_3$ (8a)
X = H; $R_1 = CD_3$; $R_2 = CH_3$ (8b)
X = D; $R_1 = CD_3$; $R_2 = CD_3$ (8c)

$R_1 = CH_3$; $R_2 = CD_3$ (2a)
$R_1 = CD_3$; $R_2 = CH_3$ (2b)
$R_1 = CD_3$; $R_2 = CD_3$ (2c)

Scheme 2

Synthesis of 2,3 - dimethoxy[$^2H_6$] - 5 - methyl - 1,4 - benzoquinone (2b) and 2,3 - dimethoxy[$^2H_6$] - 5 - methyl[$^2H_3$] - 1,4 - benzoquinone (2c)

The synthetic route for these analogs comprises the same steps as outlined above. Dimethyl[$^2H_6$]– sulfate was used for the alkylation of 3,4,5 – trihydroxybenzoic acid. The product 3,4,5 – trimethoxy[$^2H_9$] – benzoic acid (4a) was converted into the quinone (2b) via (9a), (6a), (7a) and (8b) as summarized in scheme 2. Proton NMR revealed a slight reduction of the intermediates resulting in the formation of (4a) (about 4.5%).

Reduction of (7a) using sodium borohydride–[$^2H_4$] as the reducing agent finally leads to the fully deuterated quinone (2c).

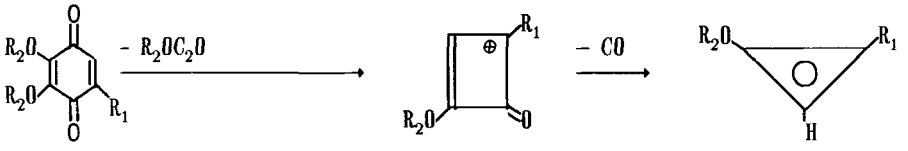
Mass spectrometry of (2), (2a), (2b) and (2c)

The mass spectrum of (2) has been partially interpreted in a previous publication (30). This provides a basis for the assignment of some mass peaks of the deuterated analogs as shown in table 1. A detailed analysis can be given here using the labeling pattern of the deuterated analogs.

The mass numbers are shifted according to the number of deuterons in the respective fragments of compounds (2), (2a), (2b) or (2c) (table 1). It is known (31) that benzoquinones give m/e = M+2 (peak no. 1 in table 1). Peak no. 2 is the molecule peak. Peak no. 3 can be attributed to the loss of a methyl group which derives from the methoxy substituents (M^+–15 in (2a), M^+–18 in (2b) and (2c)). Release of a CO fragment then leads to peak no. 5. Peak no. 4 is obviously due to the loss of a formyl group which also originates from the methoxy substituents.

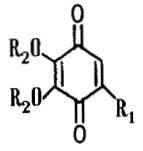
Comparing the m/e values of the peaks no.6 in the labelled and unlabelled compouds (the base peak) reveals that the corresponding fragments derived from (2b), (2a) and (2c) contain three, one and four deuterons, respectively. This observation can be conclusively attributed to a combined loss of a methyl group and formaldehyde from the methoxy moieties only.

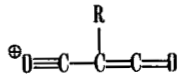
The assignment of peaks no. 7 and no. 8 to a ring fragmentation (30) followed by release of a CO – molecule could be corroborated by the shifted masses as shown in fig. 3. Likewise, the ring fragmentation as outlined in fig. 4 (30) agrees with the m/e – values found for peak 9.



(2) (R_1 = CH_3, R_2 = CH_3):	182	111	83
(2a) (R_1 = CD_3, R_2 = CH_3):	185	114	86
(2b) (R_1 = CH_3, R_2 = CD_3):	188	114	86
(2c) (R_1 = CD_3, R_2 = CD_3):	191	117	89

Figure 3: Fragmentation of (2), (2a), (2b) and (2c) (30). Numbers are the m/e – values of the corresponding ions.





(2) and (2a): $R_2 = CH_3$, R = H, m/e = 69

(2b) and (2c): $R_2 = CD_3$, R = D, m/e = 70

for R_1 cf. Fig. 2

Figure 4: An alternative fragmentation leads to the ion $C_3RO_2^+$ (30). After cleavage of the quinone ring and loss of R_2-CH_2O (from (2a), $R_2 = CH_3$) or CD_2O (from (2b) and (2c), $R_2 = CD_3$) is eliminated.

Table 1 (cf. figs. 3 and 4)

Peak	m/e (2)	m/e (2a)	m/e (2b)	m/e (2c)	M ± m	Assignment
1	184	187	190	193	$M^+ + 2$	$M^+ + 2$ – Peak
2	182	185	188	191	M^+	molecular peak
3	167	170			$M^+ - 15$	$M^+ - CH_3$
3			170	173	$M^+ - 18$	$M^+ - CD_3$
4	153	156			$M^+ - 29$	$M^+ - CHO$
4			158	161	$M^+ - 30$	$M^+ - CDO$
5	139	142			$M^+ - 43$	$M^+ - CH_3 - CO$
5			142	145	$M^+ - 46$	$M^+ - CD_3 - CO$
6	137	140			$M^+ - 45$	$M^+ - CH_3 - CH_2O$
6			138	141	$M^+ - 50$	$M^+ - CD_3 - CD_2O$
7[a]	111	114			$M^+ - 71$	$M^+ - C_3H_3O_2$
7[a]			114	117	$M^+ - 74$	$M^+ - C_3D_3O_2$
8[a]	83	86			$M^+ - 99$	$M^+ - C_3H_3O_2 - CO$
8[a]			86	89	$M^+ - 102$	$M^+ - C_3D_3O_2 - CO$
9[a]	69				$M^+ - 113$	$M^+ - CH_3 - CH_2O - C_4H_4O$
9[a]		69			$M^+ - 116$	$M^+ - CH_3 - CH_2O - C_4HD_3O$
9[a]			70		$M^+ - 118$	$M^+ - CD_3 - CD_2O - C_4H_4O$
9[a]				70	$M^+ - 121$	$M^+ - CD_3 - CD_2O - C_4HD_3O$

[a] cf. Ref. (30)

Experimental

Solvents were purified using customary procedures and stored over molecular sieve (3 Å) except for THF which was stored over sodium wire. Ethylchloroformate and triethylamine were destilled before use. Triethylamine was stored over KOH.

3,4,5 – trihydroxybenzoic acid was recrystallized twice from water in the presence of charcoal. The degree of deuteration in sodium borohydride – [$^2H_4$] (Fluka) and in dimethyl [$^2H_6$] – sulfate (Riedel–de Häen) was 99%

^1H – NMR spectra were recorded in $CDCl_3$ with a Jeol FX90–Q spectrometer. TMS was used as an internal standard. IR spectra were obtained in KBr pellets with a Bruker IFS 45 spectrometer. Electron impact mass spectra were obtained at 70 eV using a Kratos MS 80 RFA spectrometer. Thin layer chromatography was peformed on silica gel from Merck containing a fluorescence indicator (Kieselgel 60, F_{254}).

Analytical data are given in table 2. The melting points in table 2 were not corrected.

3,4,5 - Trimethoxybenzoic acid (4) and 3,4,5 - Trimethoxy[$^2H_9$] – benzoic acid (4a)

3,4,5 – Trihydroxybenzoic acid (3) was alkylated with dimethylsulfate in an alkaline medium basically following ref. 23. A stream of nitrogen was bubbled through an aqueous sodium hydroxide solution for 45 min before addition of (3). This precaution avoids oxidative decomposition and leads to a product which can be used without further purification.

The synthesis of the deuterated compound (4a) was performed analogously. Compound (3) (7.46 g; 39.66 mmol) was dissolved in a solution of 12.03 g (0.3 mol) of sodium hydroxide in water and two 10 ml portions of [$^2H_6$] – dimethylsulfate (27.8 g; 210.46 mmol) were added as described above. The methyl ester which forms as a by–product was destroyed by refluxing the reaction mixture for two hrs after addition of 3 g of sodium hydroxide in 4.5 ml of water. For analytical purposes the product was recrystallized from water. Total yield, 7.6 g (86.6 %).

2 - Iodo - 3,4,5 - trimethoxybenzoic acid (9) and 2 - iodo – 3,4,5 - trimethoxy[$^2H_9$] *- benzoic acid (9a)*

3,4,5 – Trimethoxybenzoic acid (5 g; 23.6 mmol), mercuric (II) acetate (8 g; 25 mmol) and 1.5 ml acetic acid were refluxed in 100 ml of water. After 48 hrs a white, powdery suspension was obtained. A solution of iodine (7.5 g; 29.5 mmol) and sodium iodide (15 g; 90.4 mmol) in 25 ml of water was added within 20 min to the boiling reaction mixture. The suspension which turned brownish was further heated for 4 hrs. Excess iodine was reduced by addition of solid sodium sulfite (Na_2SO_3) until the mixture retained a light brown color. After filtration of the hot solution addition of 25 ml of HCl (37 %) led to precipitation of the product. The mixture was cooled on ice for one hr and the precipitate was filtered, thoroughly washed with water and dried over KOH. The product which was obtained as colorless needles was used without further purification. For analytical purposes it was recrystallized from water. Total yield, 6.14 g (77.1 %).

The deuterated compound (9a) was obtained analogously, except that (4a) was used as the starting material (3.8 g; 17.17 mmol). After reaction with mercuric (II) acetate (5.8 g; 18.2 mmol) and 0.75 ml acetic acid in 50 ml water and after addition of iodine (5.35 g; 21.1 mmol) and potassium iodide (10.7 g; 64.5 mmol) in 20 ml water workup as described above yielded 4.57 g of (9a) (76.6 %).

2 - Hydroxy - 3,4,5 - trimthoxybenzoic acid (6) and 2 - hydroxy - 3,4,5 - trimethoxy[$^2H_9$] *- benzoic acid (6a)*

The compounds (6) and (6a) were prepared basically according to ref. 28. A solution of 10 ml of 3 M NaOH was carefully deaerated for 20 min by a stream of nitrogen. Compound (9) (2.53 g; 7.5 mmol) and copper powder (20 mg) were added and the suspension was heated at about 90^0 C for one hour. The fluffy, greenish precipitate was filtered and washed with 2 M NaOH. The product crystallized within 20 min after acidification of the filtrate with 2 N HCl. After one hour the almost white crystals were sucked off, thoroughly washed with water and dried over KOH. Total yield, 1.3 g (76 %).

The deuterated analog (6a) was obtained accordingly. Starting with 2.07 g (5.96 mmol) of (9a) the total yield of (6a) was 1.1 g (77.5 %).

Notes:

The crystalline product is obtained as a monohydrate. Complete removal of water of crystallization is necessary since water interferes with the following steps.

Small amounts (4.5 %) of (4) or (4a) were formed by reduction of (6) or (6a). Purification of the desired product was only achieved by repeated recrystallization from water or from a mixture of cyclohexane and toluene. Attempts to remove the by product chromatographically failed since (6) decomposes on silica. Thus the mixture obtained was used in the following synthesis without purification .

2,3,4 - Trimethoxy - 6 - methyl[$^2H_3$] – phenol (8a), 2,3,4 - trimethoxy[$^2H_9$] - 6 - methylphenol (8b) and 2,3,4 - trimethoxy[$^2H_9$] - 6 - methyl[$^2H_3$] - phenol(8c)

For the synthesis of (8a) the crude product (6) of the preceding step (1.36 g; ca. 5.9 mmol) and triethylamine (1.42 g, 2 ml; 14 mmol) were dissolved in 20 ml tetrahydrofurane. Ethoxycarbonylchloride (1.52 g, 1.35 ml; 14 mmol) was added dropwise over a 50 min period keeping the temperature in the reaction vessel below 5°C. After standing for 45 min at 0°C the precipitate was filtered off and washed three times with 10 ml portions of tetrahydrofurane. The filtrate was added dropwise over 45 min at 10°C to a solution of sodium borohydride – [$^2H_4$] (1 g; 23.9mmol) in 20 ml of water.

After stirring at room temperature for 3 hrs the turbid solution was acidified with 2 M HCl and extracted six times with 25 ml portions of toluene. The combined organic phases were extracted with six portions of 1 M NaOH (25 ml each). The aqueous extracts were acidified with 2 M HCl and extracted six times with 25 ml portions of toluene. The combined toluene solutions were washed with water and with 5% aqueous $NaHCO_3$. After drying ($MgSO_4$) the solvent was evaporated. A slightly yellow oil was obtained giving a total yield of 0.97 g (81.5 %). The product could be used without further purification.

The synthesis of (8b) was accomplished analogously. 1.12 g (9a) (crude product, ca. 4.7 mmol) were reacted with 1.08 g (10.62 mmol; 1.47 ml) triethylamine, 1.15 g (10.62 mmol; 1.02 ml) ethylchloroformiate and 0.89 g (23.6 mmol) sodium borohydride as described above. A slightly yellow oil (0.77 g; 78.8 %) was obtained, which was used without further purification.

The labelled compound (8c) was obtained from 1.22 g (9a) (crude product, ca. 5.1 mmol), 1.21 g (12.03 mmol; 1.66 ml) triethylamine 1.30 g (12.03 mmol; 1.15 ml) ethylchloroformiate and 1 g (23.9 mol) sodium borohydride –[$^2H_4$] under the same conditions as described above. The slightly yellow oil (0.89 g; 83 %) was used without further purification.

2,3 - Dimethoxy - 5 - methyl[$^2H_3$] *- 1,4 - benzoquinone (2a), 2,3 - dimethoxy*[$^2H_6$] - 5 - *methyl - 1,4 - benzoquinone (2b) and 2,3 - dimethoxy*[$^2H_6$] – 5 –*methyl*[$^2H_3$] *- 1,4 - benzoquinone (2c)*

A solution of 0.97 g (4.8 mmol) of (8a) in 15 ml of toluene was added to an aqueous solution of $FeCl_3$*$6H_2O$ (6 g in 20 ml of water). After vigorous stirring at room temperature for 18 hrs the toluene layer was separated and the aqueous phase was extracted five times with 10 ml portions of toluene. The combined toluene solution was dried ($MgSO_4$) and evaporated. The product was obtained as a dark red oil which crystallized spontaneously. Further purification was achieved by sublimation at 40^{0}C/0.4 mbar. Yield, 0.73 g (81.9 %).

The intermediates (8b) (0.77 g; 9.71 mmol) and (8c) (0.89 g; 4.23 mmol) were oxidized under the same conditions as above yielding (2b) (0.57g; 81.7 %) and (2e) (0.66 g; 82.3 %) respectively.

Table 2

Analytical Data

	(4)	(4a)	(9)
$^1H(CDCl_3)$	3.90(s,9H);7.35(s,2H); 10.27(br,1H)	7.38(s,2H);10.28(br,1H);	3.88(s,3H);3.90(s,3H); 3.95(s,3H);7.45(s,1H); 10.9(br,1H)
IR(KBr) (cm^{-1})	ν(C=O):1685(s);	ν(C–D):2074(m); ν(C=O):1685(s)	ν(C=O):1700(s);
MS	212(M^+);197(M^+–CH_3); 169(M^+–CH_3–CO); 141(M^+–CH_3–2CO)	221(M^+);203(M^+–CD_3); 175(M^+–CD_3–CO); 147(M^+–CD_3–2CO)	338(M^+);323(M^+–CH_3); 211(M^+–I)
R_f	(MeOH/Toluene,2:3)0.63; (Pyridin/Ethylacetate/ Toluene,1:2:2)0.43; ($CHCl_3$/MeOH,7:5)0.68	see (4)	($CHCl_3$/MeOH,7:5)0.48; (Pyridin/Ethylacetate/ Toluene,1:2:2)0.29
M.P. (°C)	168–170	167–169	151–152

	(9a)	(6)	(6a)
$^1H(CDCl_3)$	7.42(s,1H);10.8(br,1H)	3.85(s,3H);3.93(s,3H); 4.04(s,3H);7.16(s,1H); 10.28(br,1H);11.31 (br,1H)	7.13(s,1H);10.25(br,1H); 11.28(br,1H)
IR(KBr) (cm^{-1})	ν(C–D):2072(m); ν(C=O):1700(s)	ν(C=0):1647(s)	ν(C–D):2070(m); ν(C=O):1646(s)
MS	347(M^+);329(M^+–CD_3); 220(M^+–I)	228(M^+);210(M^+–H_2O); 184(M^+–CO_2);167(M^+–H_2O–CH_3–CO)	237(M^+);219(M^+–H_2O); 193(M^+–CO_2);173 (M^+–H_2O–CD_3–CO)
R_f	see (9)	(MeOH/Toluene,2:3)0,30; (Pyridin/Ethylacetate/ Toluene,1:2:2)0,10	see (6)
M.P. (°C)	151–153	103–105	103–105

Table 2 (continued)

	(8a)	(8b)	(8c)
$^1H(CDCl_3)$	3.79(s,3H);3.86(s,3H); 3.94(s,3H);5.45(s,1H); 6.43(s,1H)	2.20(s,3H);544(s,1H); 6.42(s,1H)	5.44(s,1H);6.42(s,1H)
IR(KBr) (cm^{-1})	ν(C=C):1501(s)	ν(C–D):2070(m); ν(C=C):1494(s)	ν(C–D):2070(m); ν(C=C):1494(s)
MS	201(M^+);186(M^+–CH_3); 158(M^+–CH_3–CO); 143(M^+–$2CH_3$–CO)	207(M^+);189(M^+–CD_3); 161(M^+–CD_3–CO); 143(M^+–$2CD_3$–CO)	210(M^+);192(M^+–CD_3); 164(M^+–CD_3–CO), 146(M^+–$2CD_3$–CO)
R_f	($CHCl_3$/Ether, 5:1)0.53	see (8a)	see (8a)
M.P. (°C)	oil	oil	oil

	(2a)	(2b)	(2c)
$^1H(CDCl_3)$	3.99(s,3H);4.02(s,3H); 6.43(s,1H)	2.03(d,3H);6.43(qu,1H) J=1.47Hz	6.43(s)
IR(KBr) (cm^{-1})	ν(C=O):1670(s);1656(s); ν(C=C):1604(s)	ν(C–D):2075(m); ν(C=O):1670(s); ν(C=C):1601(s)	ν(C–D):2075(m); ν(C=O):1668(s),1658(s); ν(C=C):1599(s)
MS	see table 1	see table 1	see table 1
R_f	(Toluene/MeOH,4:1) 0,66	see (2a)	see (2c)
M.P. (°C)	57–59	58–60	58–60

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