An improved synthesis of *m*-diazirinophenol

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The published synthesis of phenolic diazirine was shown to result in products which were chlorinated during the hypochlorite oxidation while the desired unchlorinated product was lost during the usual work-up. A superior synthesis is described: inclusion of pyridine during the oxidation step prevents the chlorination; the desired volatile diazirine was isolated in good yield using silica gel and reverse phase high pressure liquid chromatography.

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On a démontré que la méthode proposée dans la littérature pour la préparation de diazirine phénolique conduit d'une part, à la formation de produits qui ont été chlorés au cours de l'oxydation par l'hypochlorite alors que d'autre part, le produit non chloré désiré est perdu lors de la purification par les méthodes usuelles. On propose une synthèse supérieure qui implique l'inclusion de pyridine au cours de l'étape d'oxydation afin de prévenir la chloration; on a isole la diazirine volatile désirée avec de bons rendements en faisant appel à des chromatographies liquide à haute pression avec phase inversée et sur gel de silice.

[Traduit par le journal]

Introduction

The phenolic diazirine group was introduced (1) as a potential photoactivable biological probe and has been incorporated into a number of biological systems (2–6). It has recently been attached to the ω-position in a series of tritiated fatty acids and these have been used to identify integral membrane proteins (6, 7) and to characterize the regions of such proteins which are buried within the hydrocarbon region of the bilayer (7). Further, we have shown that these fatty acid analogues can be biologically attached to the recently discovered (8, 9) membrane proteins which are normally acylated by fatty acids. These fatty acids thus afford photoaffinity derivatives of such membrane proteins (6, 7). Consequently, the *m*-diazirinophenolic group has a wide range of biological applications.

In the course of our synthesis of this probe by the published procedures (1, 6), we found that the yield of the desired diazirine was very low and that the products obtained were mostly chlorinated isomers. It had been suggested earlier (10) that removal of HCl generated during hypochlorite oxidations prevents the formation of chlorine and thus avoids chlorination. We therefore report a superior synthesis of the *m*-diazirinophenol, using pyridine to remove the generated HCl as the pyridinium hydrochloride salt.

Experimental

General

Dimethyl formamide (DMF) and methanol were hplc grade. The petroleum ether fraction used was the one boiling between 30–60°C. All other solvents used were reagent grade. Technical grades of both *m*-hydroxybenzaldehyde and chloromethyl methyl ether were purchased from Aldrich. *tert*-Butyl hypochlorite was freshly prepared and assayed as described (11). Thin layer chromatography (tlc) was carried out on silica gel using the following solvent systems: system A, diethyl ether – petroleum ether 1:1 (v/v); system B, diethyl ether – petroleum ether 1:4 (v/v). Elemental analyses were performed by Galbraith Laboratories, Knoxville.

Methoxymethylene ether of m-*hydroxy benzaldehyde (2)*

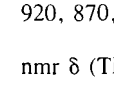
The *m*-hydroxy benzaldehyde (0.50 mol) was reacted at 0°C with 1.1 equivalent of sodium hydride in suspension in DMF. When the evolution of H_2 had stopped, an excess (0.55 mol) of chloromethyl methyl ether was added dropwise. The mixture was stirred at 0°C for 2 h and the resulting methoxymethylene ether obtained by vacuum distillation (62 g; 75%); bp 125–127°C at 4 Torr (1 Torr = 133.3 Pa); tlc: R_f 0.44 in system A; nmr δ (TMS): 3.23 (s, 3H, CH_3—O—), 4.97 (s, 2H, —O—CH_2—O—), 6.87–7.27 (m, 4H, aromatic), 9.53 (s, 1H, —C(=O)—H).

*1,3,5-Tri-(*m-*methoxymethylene phenyl ether)-2,4,6-triazabicyclo-[3.1.0]hexane (4)*

Chloramine was prepared by the cautious addition of *tert*-butyl hypochlorite (0.33 mol) to 10 *N* methanolic ammonia (280 mL) in a flask cooled by Dry Ice. The *m*-methoxymethylene benzaldehyde (**2**, 50 g) was added and the solution allowed to warm to room temperature during a reaction time of 36 h. The ammonia was allowed to evaporate and the solvent was removed by rotary evaporation under reduced pressure. The oily product was used directly without further characterization.

*Synthesis of 3-(*m-*methoxymethylene ether phenyl)-3*H*-diazirine in the absence of pyridine*

The triazabicyclohexane (**4**) prepared as described above starting with 30 g (0.18 mol) of the benzaldehyde (**2**) was an oily residue which was immediately dissolved in 40 mL of methanol and brought to 0°C. A solution of *tert*-butyl hypochlorite (0.09 mol) in *tert*-butyl alcohol (16 mL) was added dropwise and the reaction allowed to proceed for 2 h. Aliquots of the mixture were analyzed on hplc using a μ Bondapak C_{18} column with a solvent system consisting of 65% methanol–water. The mixture was poured into 1 L of a 10% solution of sodium metabisulfite and extracted 4 times with an equal volume of petroleum ether. The extracts were combined, subjected to silica gel chromatography using petroleum ether, and the fractions having absorbance at 360 nm were pooled. Evaporation of the ether afforded a yellow–green oil (1.95 g). The hplc analysis described in Fig. 2(a) identified products **5***a*, **5***b*, **5***c*, and **5***d* having retention times of 7.7, 11.5, 14.4, and 17.5 min respectively. Each of these components was obtained in pure form by preparative hplc on a μ Bondapak C_{18} column isocratically eluted with 55% methanol in water.

The peak eluting at 7.7 min (product **5***a*) was the desired diazirine (240 mg; 2%); ν_{max} (neat): 2930, 2860, 1605, 1580, 1490, 1450, 1400, 1350, 1320, 1278, 1250, 1210, 1150, 1080, 1030, 1000, 990, 920, 870, 770, 690 cm^{-1}; vis (cyclohexane), λ_{max}: 358 nm (ε 360); nmr δ (TMS): 1.98 (s, 1H, ), 3.38 (s, 3H, CH_3—O—), 5.03 (s, 2H, —O—CH_2—O—), 6.37–6.53 (m, 2H, aromatic),

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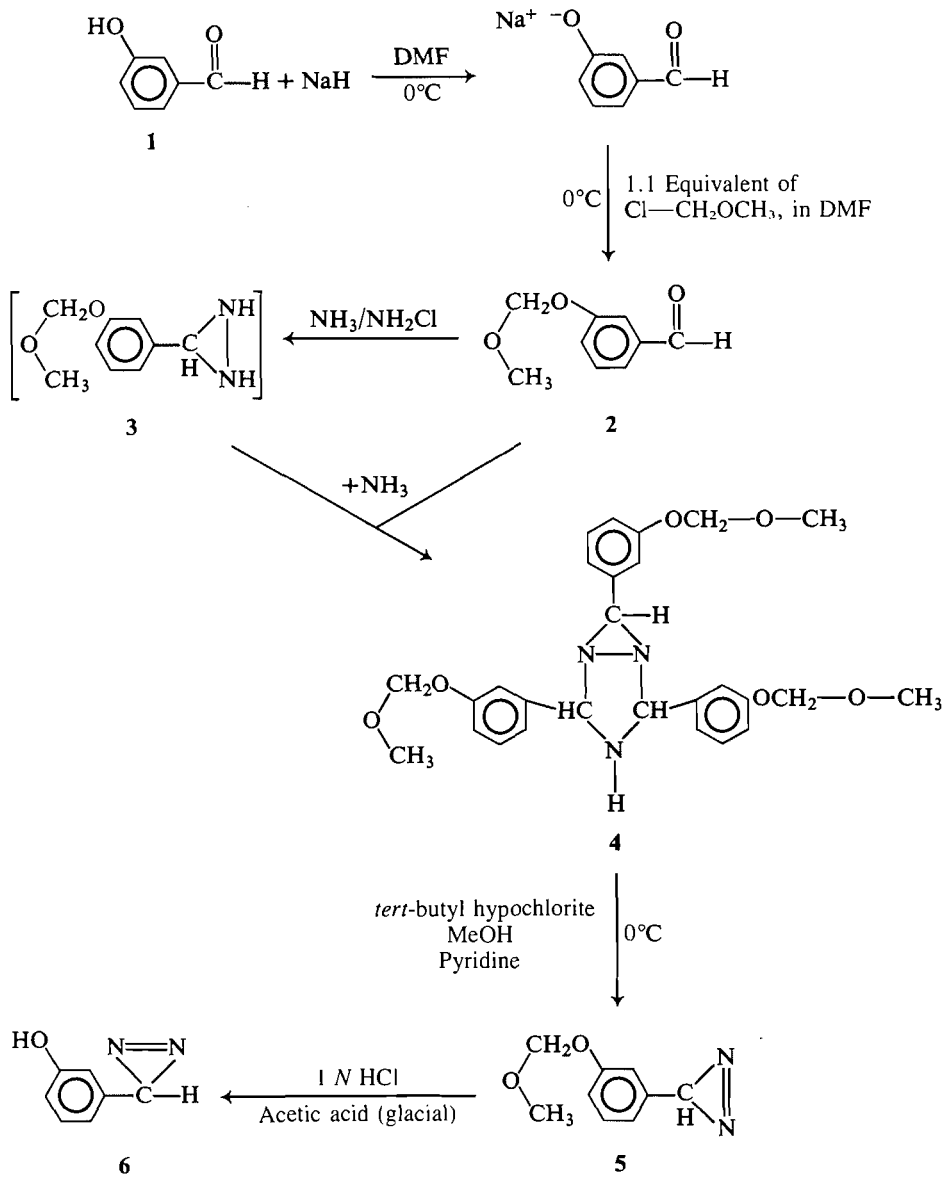
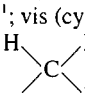
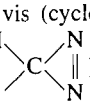


FIG. 1. Scheme for the synthesis of *m*-diazirinophenol.

6.83–7.23 (m, 2H, aromatic).

The peak observed on hplc, at 11.5 min (product **5***b*) was recovered as an oil (560 mg; 4.4%) and shown to be a chlorinated product; ν_{max} (neat): 2960, 2830, 1620, 1600, 1580, 1489, 1430, 1400, 1347, 1290, 1250, 1210, 1175, 1150, 1085, 1050, 1010, 980, 920, 815, 780, 750, 725, 625 cm^{-1}; vis (cyclohexane), λ max: 358 (ε 350); nmr δ (TMS): 2.03 (s, 1H, ), 3.52 (s, 3H, CH_3—O—), 5.20 (s, 2H, —O—CH_2—O—), 6.44 (dd, 1H, J_{ortho} = 8.2 Hz, J_{meta} = 2.0 Hz, aromatic), 6.75 (d, 1H, J_{meta} = 2.0 Hz, aromatic), 7.29 (d, 1H, J_{ortho} = 8.2 Hz, aromatic). *Anal.* calcd. for $C_9H_9N_2ClO_2$: C 50.83, H 4.27, N 13.17, Cl 16.67, O 15.05; found: C 51.27, H 4.70, N 11.20, Cl 17.78, O 14.86.

The peak eluting from hplc at 14.4 min (product **5***c*) was recovered as an oil (270 mg; 2.1%) and shown to be a chlorinated product; nmr δ (TMS): 2.54 (s, 1H, ), 3.50 (s, 3H, CH_3—O—), 5.24 (s, 2H, —O—CH_2—O—), 6.79 (dd, 1H, J_{ortho} = 6.8 Hz, J_{meta} = 2.5 Hz, aromatic), 7.06 (m, 2H, J_{ortho} = 6.8 Hz, J_{ortho} = 8.3 Hz, J_{meta} = 2.5 Hz, aromatic).

The peak observed on hplc at 17.5 min (product **5***d*) was recovered as an oil (700 mg; 5.5%) and shown to be a chlorinated product; ν_{max} (neat): 2930, 2860, 2830, 1620, 1600, 1571, 1475, 1425, 1400, 1345, 1305, 1278, 1238, 1210, 1170, 1150, 1140, 1080, 1060, 1010, 920, 870, 810, 640 cm^{-1}; vis (cyclohexane), λ_{max}: 358 (ε 289); nmr δ (TMS): 2.45 (s, 1H, ), 3.39 (s, 3H, CH_3—O—), 5.02 (s, 2H, —O—CH_2—O—), 5.90 (d, 1H, J_{meta} = 2.9 Hz, aromatic), 6.88 (dd, 1H, J_{ortho} = 8.8 Hz, J_{meta} = 2.9 Hz, aromatic), 7.25 (d, 1H, J_{ortho} = 8.8 Hz, aromatic). *Anal.* calcd. for $C_9H_9N_2ClO_2$: C 50.83, H 4.27, N 13.17, Cl 16.67, O 15.05; found: C 51.86, H 4.86, N 11.25, Cl 17.05, O 15.27.

*Synthesis of 3-(*m*-methoxymethylene ether phenyl)-3*H*-diazirine in the presence of pyridine*

The triazabicyclohexane (**4**), prepared as described above starting with 50 g (0.33 mol) of the benzaldehyde (**2**) was dissolved in 60 mL of methanol and brought to 0°C. After addition of pyridine (0.51 mol), *tert*-butyl hypochlorite (0.17 mol in 20.0 mL of *tert*-butyl alcohol) was added dropwise over a period of 10 min. The reaction was allowed to proceed for 2 h. The mixture was then processed as described above

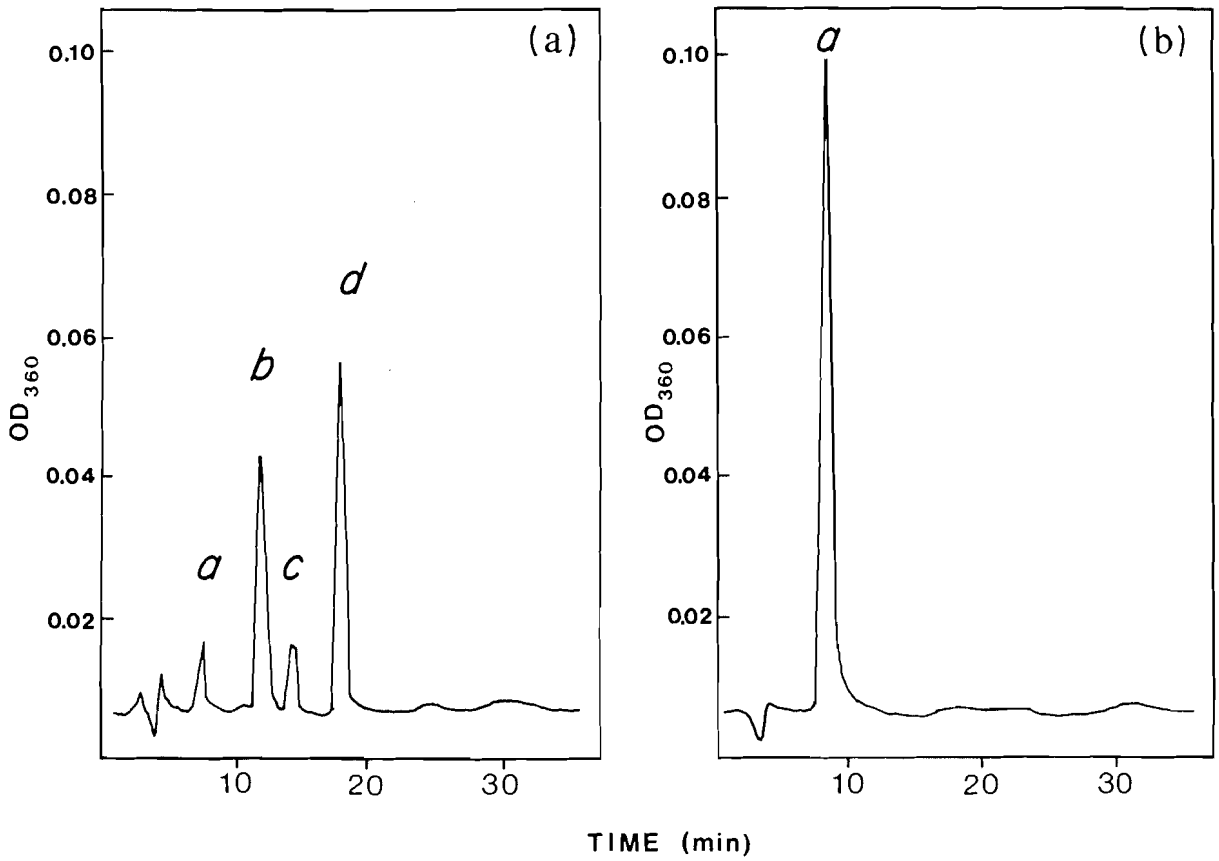
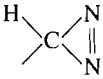


FIG. 2. High pressure liquid chromatographic analyses of the oxidation products (**5**) in the absence (a) and the presence (b) of pyridine. The reaction conditions were as described in the experimental section for the oxidation of the triazabicyclohexane. An aliquot (10 μL) of the reaction mixture was injected after 45 min, on a C_{18} reverse-phase hplc column using 65% methanol in water as an eluting solvent. The eluates were monitored using an ISCO detector equipped with a 340–365 nm filter.

for the oxidation of the triazabicyclohexane (**4**) in the absence of pyridine. Final purification was achieved by preparative hplc on a μ Bondapak C_{18} column using 55% methanol–water as solvent. After extraction into diethyl ether and drying, a pale yellow–green oil was obtained (4.30 g; 24%); tlc: R_f 0.35 in system B; ν_{max} (neat): 2930, 2860, 1605, 1580, 1490, 1450, 1400, 1350, 1320, 1278, 1250, 1210, 1150, 1080, 1030, 1000, 990, 920, 870, 770, 690 cm^{-1}; vis (cyclohexane), λ_{max}: 358 nm (ε 360); nmr δ (TMS): 1.98 (s, 1H, ), 3.38 (s, 3H, CH_3—O—), 5.03 (s, 2H, —O—CH_2—O—), 6.37–6.53 (m, 2H, aromatic), 6.83–7.23 (m, 2H, aromatic). *Anal.* calcd. for $C_9H_{10}N_2O_2$: C 61.01, H 5.12, N 15.81, O 18.06; found: C 61.25, H 6.46, N 14.45, O 17.82, Cl none.

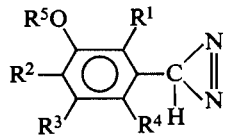
m-*Diazirinophenol* (*6*a)

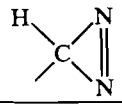
The procedure was adapted from the one described (2). The dry diazirine (*5a*) (17 mmol) was dissolved in glacial acetic acid (51 mL); 1 *N* aqueous HCl (9.58 mL) was added and allowed to react for 2 h. The mixture was then transferred to 1.8 L of 1 *N* sodium bicarbonate and the crude *m*-diazirinophenol isolated by extraction into diethyl ether. The pure *m*-diazirinophenol generated was isolated by column chromatography on silica gel using petroleum ether and diethyl ether (95:5, v/v), followed by preparative hplc using an isocratic solvent system (25% methanol in water). The resulting unchlorinated phenolic diazirine was obtained as an oil (3.0 g, 90% yield); vis (cyclohexane), λ_{max}: 358 nm (ε 360); nmr δ (TMS): 1.98 (s, 1H, ), 5.47 (s, broad, 1H, arom-OH), 6.32 (dd, 1H, J_{meta} = 1.6 Hz, J_{meta} = 2.6 Hz, aromatic), 6.49 (ddd, 1H, J_{ortho} = 7.7 Hz, J_{meta} = 1.0 Hz, J_{meta} = 1.7 Hz, aromatic), 6.76 (ddd, 1H, J_{ortho} = 8.1 Hz, J_{meta} = 1.0 Hz, J_{meta} = 2.6 Hz, aromatic), 7.16 (t, 1H, J_{ortho} = 7.9 Hz, aromatic). *Anal.* calcd. for $C_7H_6N_2O$: C 62.88, H 4.51, N 20.88, O 11.93; found: C 61.98, H 4.36, N 16.40, O 13.13, Cl none.

Results and discussion

Our synthetic approach closely resembles that of Smith and Knowles (1) in that we form the triazabicyclohexane intermediate (**4**) prior to oxidative cleavage via *tert*-butyl hypochlorite (Fig. 1). Attempts at improving the yield of (*5a*) by other oxidation methods failed to produce a significant yield of the diazirine with *para*-substituted arylbicyclohexanes (1). The yields of the isolated *m*-diazirinophenol were typically in the range of 2–5% after oxidation of the triazabicyclohexane. We therefore studied the oxidation using hplc and the characteristic absorption at 360 nm of the diazirine to monitor the reaction, as shown in Fig. 2. The standard oxidation conditions resulted in a number of products as shown in Fig. 2(a). These products were isolated by preparative hplc and their nmr and vis spectra were determined. The small peak eluting at 7.7 min (Fig. 2(a)) contained product *5a* and had the nmr and vis characteristics expected for the desired diazirine. The products *5b*, *5c*, and *5d*, eluted at 11.5, 14.5, and 17.5 min respectively, had similar uv–vis spectra; however, their nmr spectra indicated the loss of an aromatic proton (3H aromatic for products *5b*, *5c*, and *5d*). Elemental analysis of these products was complicated by the fact that they were light- and heat-sensitive oils; however, the results unequivocally showed that these were all isomeric

TABLE 1. ^{1}H nuclear magnetic resonance shifts (δ ppm) of the chlorinated and unchlorinated diazirines



Compounds		R^5		Aromatic protons			
		CH_3—O—CH_2—	H	R^1	R^2	R^3	R^4
*5*a*	1.98	3.38 s, 3H 5.03 s, 2H	—	6.37– 7.23 (4H, m)	6.37– 7.23 (4H, m)	6.37– 7.23 (4H, m)	6.37– 7.23 (4H, m)
5*b*	2.02	3.51 s, 3H 5.22 s, 2H	—	6.75 d (J_m = 2.0)	Cl	7.29 d (J_o = 8.2)	6.44 dd (J_o = 8.2, J_m = 2.0)
5*c*	2.54	3.53 s, 3H 5.26 s, 2H	—	Cl	7.12 dd (J_o = 6.9, J_m = 2.5)	7.09 dd (J_o = 6.9, 8.3)	5.93 dd (J_o = 6.8, J_m = 2.5)
5*d*	2.45	3.39 s, 3H 5.02 s, 2H	—	5.90 d (J_m = 2.9)	6.88 dd (J_o = 8.8, J_m = 2.9)	7.25 d (J_o = 8.8)	Cl
†6*a*	1.98	—	5.42	6.32 dd (J_m = 1.6, J_m = 2.6)	6.49 ddd (J_o = 7.7, J_m = 1.0, J_m = 2.6)	7.16 t (J_o = 7.9)	6.76 ddd (J_o = 8.1, J_m = 1.0, J_m = 2.6)

*5*a*, *b*, *c*, and *d* are products from oxidation in absence of pyridine.
†6*a* is derived from oxidation in presence of pyridine.

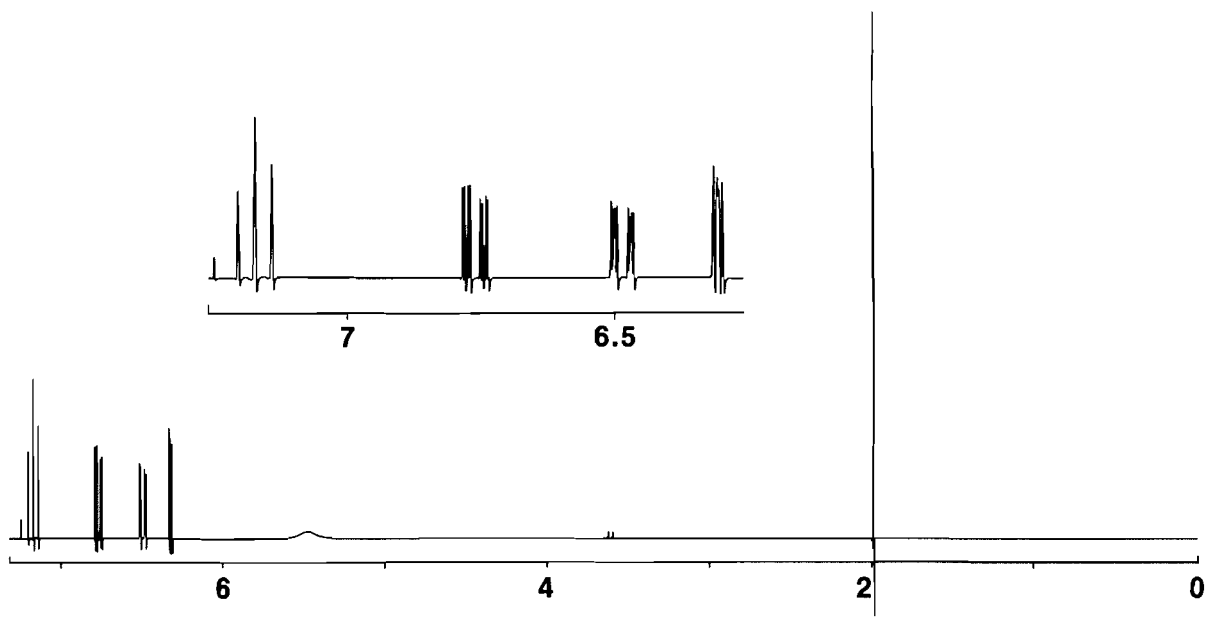


FIG. 3. The ^{1}H nmr spectrum ($CDCl_3$) of the unchlorinated *m*-diazirinophenol **6***a*.

forms of monochlorinated *m*-diazirinophenol.

Analysis of the nmr spectra using high resolution (250 MHz) nmr and spin decoupling indicated that products **5***b*, **5***c*, and **5***d* were chlorinated on the phenyl ring in positions R^2, R^1, and R^4 respectively (Table 1). It is of particular interest to note that chlorination in positions R^1 or R^4 (products **5***c* and **5***d* respectively) has a dramatic effect on the position of the adjacent diazirine proton while chlorination in position R^2 (product **5***b*) leaves this proton relatively unaffected compared to the unchlorinated compound. Furthermore the position of the *meta* proton at R^4 (when R^1 is chlorinated) or R^1 (when R^4 is chlorinated) is shifted to higher field, while the position of the proton at R^2 is shifted to lower field under these conditions. Detailed analysis of the nmr spectra of the chlorinated products enabled us to assign the position of the aromatic protons of the pure *m*-diazirinophenol (**6***a*) (see Fig. 3 for the nmr spectrum).

It was concluded that this chlorination was the result of the generation of Cl_2 during the hypochlorite oxidation. The formation of the diaziridine (**3**) generates HCl which is oxidized to Cl_2 by the hypochlorite, as had been suggested earlier (10). The pyridine traps the hydrochloric acid as the pyridinium hydrochloride salt and consequently avoids chlorination of the desired product. When the oxidation was performed in the presence of pyridine, the only diazirine-containing product

formed was eluted in the region of the desired material on hplc, as shown in Fig. 2(b); the chlorinated products **5***b*, **5***c*, and **5***d* normally eluted at 11.5, 14.5, and 17.5 min respectively were entirely absent. The pyridine thus prevented the formation of undesired products and the yield of the desired phenyldiazirine (**5***a*) was greatly improved. Removal of the methoxy methylene group afforded the pure *m*-diazirinophenol (**6***a*).

It should also be noted that the isolated yield of the unchlorinated diazirine was affected by the work-up, since it was found to be volatile while the chlorinated isomers were not. Purification by fractional distillation was avoided due to the potentially explosive properties of the diazirine; the desired pure diazirine was therefore obtained by preparative hplc, extraction into diethyl ether, and drying under nitrogen. This procedure resulted in isolated yields of 24 to 30% of the desired diazirine.

It is interesting to note that in the unchlorinated *m*-diazirinophenol, the diazirine proton is observed at δ 1.98 while the position previously reported (6) was at δ 2.4. The compound actually reported in the literature therefore likely corresponded to the major chlorinated isomer (product **5***d*, Table 1). Our previous studies used the unchlorinated *m*-diazirinophenol isolated by hplc from the crude product obtained in the absence of pyridine (5). We have shown that fatty acid analogues synthesized using this diazirine are readily incorporated into phospholipids and proteins of mammalian cells in culture (5, 7). The failure of others (12) to observe biosynthetic utilization of these fatty acids may thus be due to their use of a chlorinated isomer. In order to use these probes for biological experiments it is clearly important to avoid the chlorination.

Acknowledgements

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