Journal of Chromatography, 645 (1993) 293–301
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 142

Gas chromatographic–mass spectrometric study of the reductive silylation of hydroxyquinones

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(First received December 28th, 1992; revised manuscript received March 30th, 1993)

ABSTRACT

Reductive trimethylsilylation was carried out with N-methyl-N-trimethylsilyltrifluoroacetamide under optimized conditions to afford quantitatively the all-silylated products of several hydroxynaphthoquinones and -anthraquinones. The silylated products were investigated by GC–MS. The fragmentation patterns of the compounds provided valuable information about their structure and consequently about the structure of the initial hydroxyquinones. The silylation and GC–MS separation scheme was applied to mixtures of hydroxyquinones, leading to effective separation of all the ingredients, which included several hydroxyquinone isomers.

INTRODUCTION

Hydroxyquinones, their substituted derivatives and their transition metal chelates have long been known to possess numerous chemically and biologically significant properties [1–5]. For instance, several of the best known and widely used anthracycline and tetracycline antibiotics possess the hydroxyquinone structure, which is thought to be responsible for their biological activity [6,7]. The silylation of hydroxyquinones has attracted much attention in the last few years, because it offers the possibility of protecting the functional carbonyl and hydroxyl groups against destructive reaction conditions in a synthetic process, thus providing suitable intermediates. The products may be used as prodrugs, as their lipophilicity coupled with the facile hydrolysis of the Si–O bond would allow them to penetrate easily through lipophilic membranes and liberate the parent drug by gradual hydrolysis [8]. The conversion into their less polar and more volatile and thermally stable silyl ethers improves their GC–MS analysis and identification. Hence the GC–MS study of hydroxyquinones, both naturally occurring and synthetic, is of importance.

Despite the extensive studies of hydroxyquinones and their derivatives, little information is available about their silylation [9–12], which concerns mainly partial silylation of the hydroxyl groups of hydroxyanthraquinones for GC analysis. Henriksen and Kjøsen [13] dealt with the reductive silylation of some hydroxyanthraquinones for GC–MS analysis. Their approach suggests prolonged reaction periods and complex reaction conditions, involving both bases and solvents.

This work is a part of a programme to develop a better understanding of silylation reactions of quinones for synthetic, analytical and biological purposes [14,15]. We report here a simple and very efficient method for the quantitative reductive silylation of both hydroxynaphtho- and hydroxyanthraquinones with N-methyl-N-tri-

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0021-9673/93/$06.00

methylsilyltrifluoroacetamide (MSTFA), along with a detailed discussion of the mass spectra of the products and a proposal for a fragmentation mechanism. The silylation scheme was applied also to synthetic mixtures of hydroxyquinones resulting in successful GC–MS separation of the silylated derivatives, which is particularly important for the isomers of dihydroxyanthraquinones.

EXPERIMENTAL

Materials

The hydroxyquinones employed were purchased from Fluka and were of analytical-reagent grade. Their purity was checked by TLC (one spot with a 20-μg sample). MSTFA was also obtained from Fluka and was of GC quality.

Reductive trimethylsilylation

Trimethylsilyl derivatives were prepared by treating of the hydroxyquinone with MSTFA. Although many silylating agents with different and specific activities are currently available, MSTFA was chosen as the most suitable among the four active silylamides [16] the others being bistrimethylsilyltrifluoroacetamide (BSTFA), bistrimethylsilylacetamide (BSA) and N-methyl-N-trimethylsilylacetamide (MSA), because of its greatly reduced retention time (t_R) (b.p. 132°C) and its volatile by-product N-methyltrifluoroacetamide (MTFA) and it was used as the reaction solvent. MTFA appears as a symmetrical peak before that of MSTFA and does not influence the chromatogram. Further, it can be easily removed by blowing a stream of dry nitrogen on to the surface of the reaction mixture at room temperature.

The extent of silylation was controlled by varying the MSTFA to hydroxyquinone ratio. The appropriate amount of NH_4I was added at the beginning (Table I). The reaction was carried out at 60°C and was completed in 20–60 min. The samples were then injected directly into the GC–MS system.

During the process of optimizing the reaction conditions (hydroxyquinone-to-MSTFA ratio, amount of NH_4I, reaction time, temperature), occasionally more than one peak was obtained in the gas chromatogram, owing to the insufficient amount of the silylating agent used. The additional peaks were due to unsilylated compound and mono-, di-, etc., silylated derivatives.

In order to avoid the formation of siloxane by-products, particular care was taken to protect the reactants and products from moisture, even in trace amounts, by thorough drying of all starting materials and apparatus.

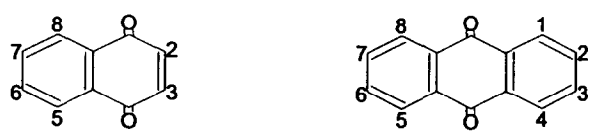
Instrumentation

GC–MS analysis was carried out on a Hewlett-Packard Model HP 5890 gas chromatograph coupled with a VG TS-250 mass spectrometer. The GC system was fitted with a 25 m × 0.2 mm I.D. OV-1 column and the end of the column was introduced directly into the mass spectrometer analyser chamber. The system was operated under the following conditions: helium pressure, 5 psi; injector temperature, 280°C; and GC column temperature, 80°C for 3 min, increased at 25°C/min from 80 to 280°C, held at 280°C for 10 min. The mass spectrometer was set to scan from 40 to 700 u per nominal second with an ionizing voltage of 70 eV.

RESULTS AND DISCUSSION

Optimization of reaction conditions

The reductive silylation reaction was applied to ten different hydroxyquinones, namely 5-hydroxy-1,4-naphthalenedione [5-HNQ (**1**)], 2-hydroxy-1,4-naphthalenedione [2-HNQ (**2**)], 5,8-dihydroxy-1,4-naphthalenedione [5,8-DHNQ (**3**)], 1-hydroxy-9,10-anthracenedione [1-HAQ



1 5-OH
2 2-OH
3 5,8-$(OH)_2$
7 5,8-$(OH)_2$-2-CH(OH)CH_2CHC$(CH_3)CH_3$
4 1-OH
5 1,4-$(OH)_2$
6 1,5-$(OH)_2$
8 1,8-$(OH)_2$
9 2-CH_2OH
10 1,2-$(OH)_2$

Fig. 1. Structures of hydroxynaphtho- and hydroxyanthraquinones.

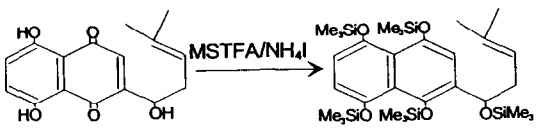


Fig. 2. Reaction scheme of the reductive silylation of hydroxyquinones **1–10**.

(**4**)], 1,4-dihydroxy-9,10-anthracenedione [1,4-DHAQ (**5**)], 1,5-dihydroxy-9,10-anthracenedione [1,5-DHAQ (**6**)], 5,8-dihydroxy-2(1-hydroxy-4-methyl-3-pentenyl)-1,4-naphthalenedione [5,8-DHHMPNQ (**7**)], 1,8-dihydroxy-9,10-anthracenedione [1,8-DHAQ (**8**)], 2-hydroxymethyl-9,10-anthracenedione [2-HMAQ (**9**)] and 1,2-dihydroxy-9,10-anthracenedione [1,2-DHAQ (**10**)] (Fig. 1) and to a mixture of the entire series.

The reductive silylation reaction may be expressed as in the reaction scheme shown in Fig. 2 for [5,8-DHHMPNQ (**7**)].

The reductive silylation reaction was applied using gradually increasing amounts of the silylating agent (MSTFA), in order to reach the optimum ratio of hydroxyquinone/silylating agent (Fig. 3). The completion of the reaction was checked by GC (one peak).

Table I presents the optimum conditions and yields of the reductive silylation reaction of the hydroxyquinones.

The longer reaction periods and the increased amounts of silylating agent required for the *ortho*

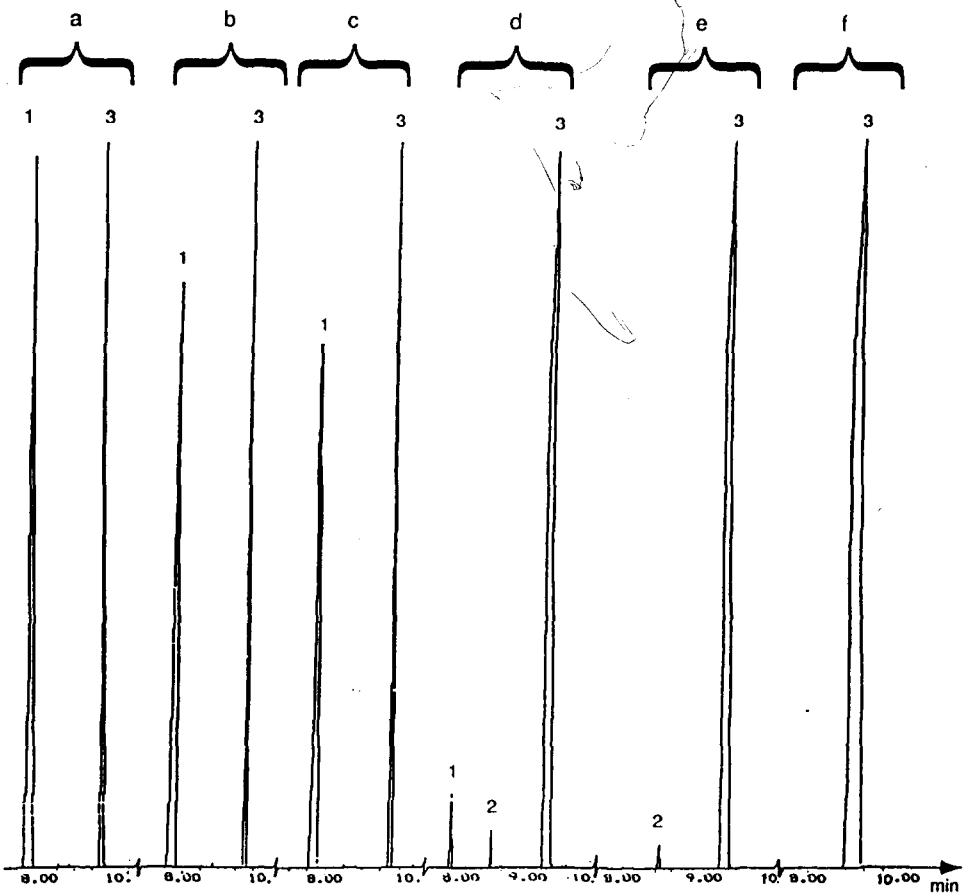


Fig. 3. 5-HNQ (**1**) as an example of optimization process of the hydroxyquinone-to-MSTFA ratio. Moles of hydroxyquinone to moles of MSTFA = (a) 1:14, (b) 1:21, (c) 1:28, (d) 1:35, (e) 1:40, and (f) 1:42. For conditions, see Experimental. Peaks: 1 = 5-HNQ-TMS; 2 = 5-HNQ-2-TMS; 3 = 5-HNQ-3-TMS.

TABLE I

REDUCTIVE SILYLATION OF VARIOUS HYDROXYQUINONES WITH MSTFA IN THE PRESENCE OF NH_4I

Substrate		Conditions				Product[a,b]		
No.	Compound	Temperature (°C)	Time (min)	Quinone/MSTFA (mmol)	MSTFA/NH_4I (ml/mg)	No.	Compound (%)	Yield[c]
1	5-HNQ	60	20	1:42	1:4	**1a**	5-HNQ-3-TMS	100
2	2-HNQ	60	20	1:42	1:4	**2a**	2-HNQ-3-TMS	100
13	5,8-DHNQ	60	30	1:56	1:4	**3a**	5,8-DHNQ-4-TMS	100
14	1-HAQ	60	25	1:42	1:12	**4a**	1-HAQ-3-TMS	100
15	1,4-DHAQ	60	60	1:80	1:12	**5a**	1,4-DHAQ-4-TMS	100
6	1,5-DHAQ	60	60	1:94	1:12	**6a**	1,5-DHAQ-4-TMS	100
7	5,8-DHHPMNQ	60	60	1:80	1:4	**7a**	5,8-DHHPMNQ-5-TMS	100
8	1,8-DHAQ	60	30	1:56	1:12	**8a**	1,8-DHAQ-4-TMS	100
9	2-HMAQ	60	25	1:56	1:4	**9a**	2-HMAQ-3-TMS	100
10	1,2-DHAQ	60	30	1:56	1:12	**10a**	1,2-DHAQ-4-TMS	100

[a] All products showed the expected spectra for the structures assigned.
[b] The silyl ether corresponding to substrate ***n*** is denoted as ***n*a**.
[c] Determined by GC.

2, **10** and *peri* (**1**, **3–8**), hydroxy-substituted naphtho- and anthraquinones should be attributed to the strong hydrogen bonds between the hydroxyl groups and the quinonoid oxygens [17].

GC separation of silylated products

GC retention and MS data for the trimethylsilylated hydroxyquinones are presented in Table II.

In contrast to the free polyhydroxyquinones [10] and in spite of the relatively high molecular masses resulting from the reductive silylation, all compounds were eluted as symmetrical peaks without tailing.

As shown in Table II, the GC elution order largely follows the molecular masses, with the exception of compounds **4a**, **7a** and **9a** in which more or less masking of the polar aromatic system resulted to reduced or increased t_R [9, 12].

MS fragmentation of trimethylsilylated products of hydroxyquinones

Few mass spectra of the trimethylsilylated hydroxyquinones have been discussed in detail before [13].

In this study of the mass spectra of TMS derivatives of the hydroxyquinones **1–10**, the ions of m/z 73, 45 and 147 are common to all of them. The ion of m/z 73 corresponds to the trimethylsilyl cation, $[SiMe_3]^+$, and a mechanism for its production has been proposed by Henriksen and Kjøsen [13]. The ion of m/z 45 has also been observed in the mass spectra of di-TMS derivatives of dihydroxy-, diamino-, etc., naphthalenes [18] and corresponds to the $[CH_5Si]^+$ cation. Finally, the ion of m/z 147 is frequently observed in the mass spectra of di-TMS derivatives and is attributed to $(CH_3)_3Si{-}O^+ = Si(CH_3)_2$, produced upon rearrangements [19–21].

The compounds under study may be classified in two categories with respect to their fragmentation pattern; one possessing *peri* (Fig. 4) and the other *ortho* silylated groups (Fig. 5).

The presence of the $[M-88]^+$ ion in abundance in all the studied mass spectra except that of **9a**, could be explained by the extreme local steric crowding leading to extensive fragmentation. Thus, elimination of a methyl group in the first step and subsequently of a trimethylsilyl radical from the molecular ion give rise to the $[M-88]^+$ ion. This fragmentation pattern is repeated for every existing pair of trimethylsilyl groups in *ortho* and *peri* positions in the molecule. The driving force for the expulsion of the trimethylsilyl radical, in the second step, is the

TABLE II

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA FOR THE REDUCTIVELY SILYLATED HYDROXYQUINONES **1–10**

No.	Hydroxyquinone	Product		t_R (min)	$M^{+\cdot}$: m/z, relative intensity (%)	Principal ions: m/z, relative intensity (%)[a,b]
		No.	Compound			
1	5-HNQ	**1a**	5-HNQ-3-TMS	8:16	392(100)	394(18), 395(28), *392(100)*, 377(8), 304(12), 289(30), 273(12), 229(5), 147(1), 73(62), 45(18)
2	2-HNQ	**2a**	2-HNQ-3-TMS	8:20	392(100)	394(16), 393(30), *392(100)*, 377(5), 304(20), 289(15), 229(10), 147(5), 73(63), 45(20)
3	5,8-DHNQ	**3a**	5,8-DHNQ-4-TMS	8:36	480(95)	482(25), 481(31), 480(95), 392(10), 304(12), 274(8), 244(1), 229(5), 147(1), *73(100)*, 45(20)
4	1-HAQ	**4a**	1-HAQ-3-TMS	9:38	442(50)	444(8), 443(19), 442(50), 354(12), 339(6), 324(3), 281(9), 279(3), 235(3), 147(1), 74(9), *73(100)*, 45(18)
5	1,4-DHAQ	**5a**	1,4-DHAQ-4-TMS	9:45	530(7)	530(7), 442(2), 354(5), 324(2), 294(1), 279(2), 147(2), *73(100)*, 45(20),
6	1,5-DHAQ	**6a**	1,5-DHAQ-4-TMS	9:58	530(11)	530(11), 442(1), 354(6), 324(1), 294(1), 279(1), 147(1), *73(100)*, 45(20)
7	5,8-DHHMPNQ	**7a**	5,8-DHHMPNQ-5-TMS	10:07	650(5)	650(5), 583(10), 582(17), 581(40), 493(10), 421(5), 405(3), 229(1), 147(2), *73(100)*, 45(10)
8	1,8-DHAQ	**8a**	1,8-DHAQ-4-TMS	10:25	530(10)	531(5), 530(10), 442(5), 369(5), 412(1), 297(1), 279(1), *73(100)*, 45(20)
9	2-HAQ	**9a**	2-HAQ-3-TMS	10:35	456(47)	458(7), 457(20), 456(47), 367(5), 294(2), 279(7), 147(5), 75(6), 74(8), *73(100)*, 45(25)
10	1,2-DHAQ	**10a**	1,2-DHAQ-4-TMS	10:50	530(75)	532(18), 531(30), 530(75), 515(5), 442(18), 412(1), 369(6), 297(4), 279(1), 147(3), 74(8), *73(100)*, 45(20)

[a] Relative to the base peak.
[b] The base peak is italicized.

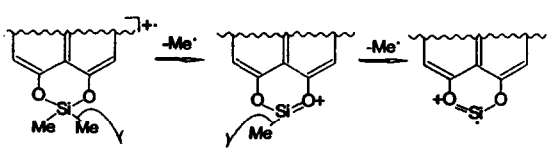


Fig. 4. Main fragmentation path of the *peri*-silylated hydroxyquinones.

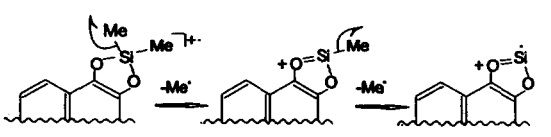


Fig. 5. Main fragmentation path of the *ortho*-silylated hydroxyquinones.

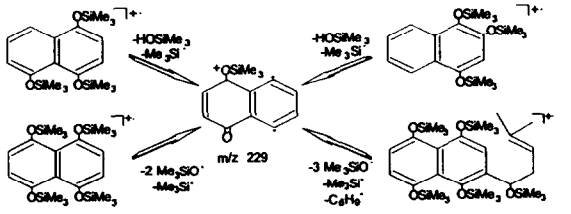


Fig. 6. Proposed fragmentation mechanism for the reductively silylated hydroxynaphthoquinones **1**, **2**, **3** and **7**.

stability of the silicon–oxygen bond, which leads to the formation of a stable cyclic radical cation. The structure of the $[M-88]^+$ ion, for both *peri* and *ortho* isomers, has been proposed [13].

According to Smith *et al.* [18], the ions $[M-103]^+$ and $[M-118]^+$, which are also observed in the mass spectra, are characteristic of the silyl ethers of *peri*-disilylated naphthalenes produced by elimination of a second and, in sequence, a third methyl radical and correspond to six-membered cationic chelate rings, of the type 2-methyl-1,3-dioxa-2-silanan and 1,3-dioxa-2-silanen, respectively (Fig. 4).

Ortho-disilylated naphthalenes also show these $[M-103]^+$ and $[M-118]^+$ ions, but to a lesser extent, owing to the reduced stability of their five-membered radical cationic ring (Fig. 5). Further, the absence or the low intensity of the previously mentioned ions, and also $[M-88]^+$ for the di-TMS derivatives of *meta*- and *para*-isomers, demonstrate that such positional isomerism *per se* is not responsible for the production of these ions in the disilylated naphthalenes.

These results are in agreement with ours (Table II). Consequently, this pronounced *ortho* and *peri* effect is valuable in the interpretation and identification of unknown hydroxyquinones in complicated mixtures or in natural products.

Two more ions of great importance are those of m/z 229 and 279. The former is characteristic of all the silylated hydroxynaphthoquinones studied and the latter characterizes all the silylated hydroxyanthraquinones. These two ions correspond to the cationic diradical of 1,4-naphthoquinone and 9,10-anthraquinone, respectively (Figs. 6 and 7).

The mass spectra of the tri-TMS ethers of **1**, **2** and **4**, exhibit a common fragmentation pattern and so do the tetra-TMS ethers **3a**, **5a**, **6a**, **8a**

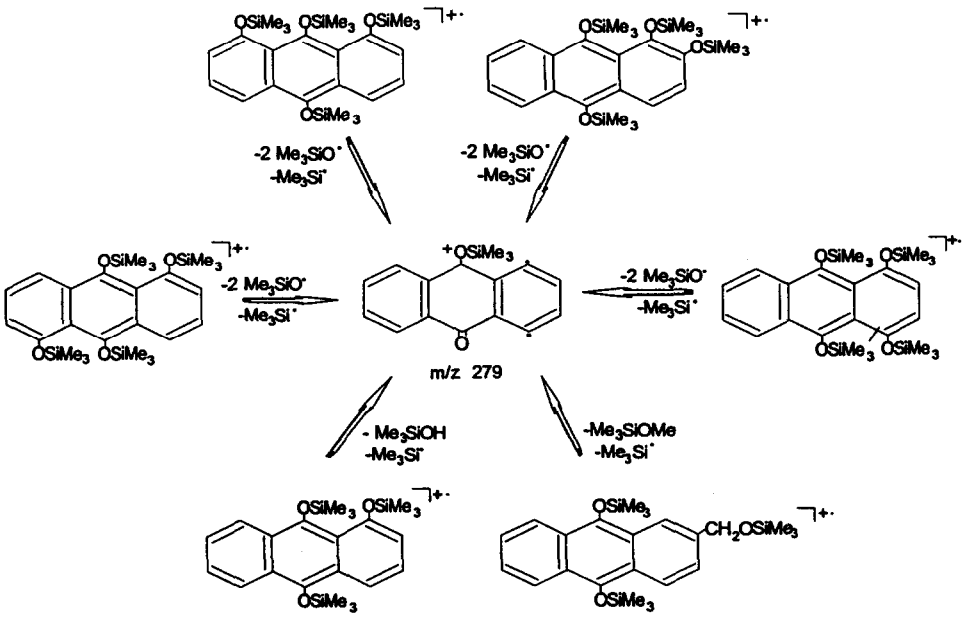


Fig. 7. Proposed fragmentation mechanism for the reductively silylated hydroxynaphthoquinones **4**, **5**, **6**, **8**, **9** and **10**.

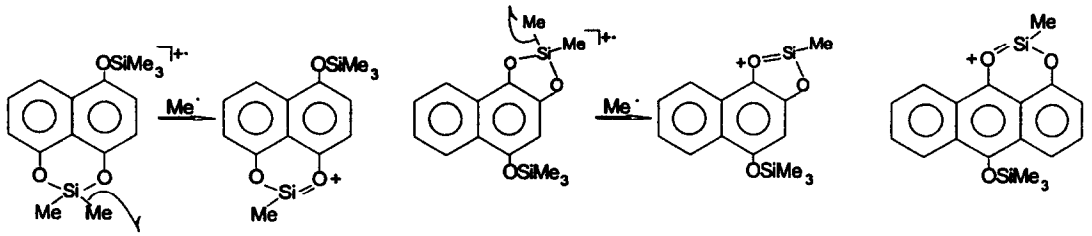


Fig. 8. Principal ions formed by the fragmentation of the reductively silylated hydroxyquinones **1** (**a**, **c**), **2** (**b**, **d**) and **4** (**e**).

and **10a**. Compounds **7a** and **9a** follow their own fragmentation pathway due mainly to the existence of an aliphatic side-chain in each of them.

Mass spectra of 5-HNQ-3-TMS (1a), 2-HNQ-3-TMS (2a) and 1-HAQ-3-TMS (4a)

Both **1a** and **2a** show the molecular ion $M^{+\cdot}$ of m/z 392 as the base peak and the $[M-88]^+$ ion, **a**, **b**, of m/z 304 in significant abundance (12% and 20%, respectively) (Fig. 8). However, owing to the *ortho* effect, the ion **c**, $[M-103]^+$, of m/z 289, is of lesser abundance (15%) in **2a** than in its isomer **1a** (**d**, 30%). In the mass spectrum of **4a** the ion $[M-103]^+$ of m/z 339, **e**, is of lesser abundance (6%) than the $[M-88]^+$ ion of m/z 354, suggesting the greater stability of the former ion, **e**.

Mass spectra of 5,8-DHNQ-4-TMS (3a), 1,4-DHAQ-4-TMS (5a), 1,5-DHAQ-4-TMS (6a), 1,8-DHAQ-4-TMS (8a) and 1,2-DHAQ-4-TMS (10a)

In the mass spectra of all these trimethylsilyl ethers the base peak is due to a fragment of m/z 73, through the gradual loss of two trimethylsilyl radicals for the first three or only one for the last two. Therefore, two peaks are observed at m/z 392 and 304 for **3a** and m/z 442 and 354 for the others, corresponding to the $[M-88]^+$ and $[M-176]^+$ ions, respectively.

For the last two ethers elimination occurs first of a tetramethylsilane molecule followed by the loss of a trimethylsilyl radical and finally of a $(CH_3)_2Si{=}CH_2$ molecule. Consequently, three ions are formed, of m/z 442 (**m** and **n**, respectively, for **8a** and **10a**), m/z 369, (**p**, **q**) and m/z 297 (**r**, **t**), the last one being of very low abundance (0.1% and 2%, respectively) (Fig. 9).

Mass spectra of 5,8-DHHPMNQ-5-TMS (7a) and 2-HMAQ-3-TMS (9a)

The fragmentation pattern of **7a** begins by the elimination of the side-chain followed by two successive losses of tetramethylsilane molecules. Three ions of m/z 581, 493 and 405 are formed, corresponding to $[M-C_5H_9]$ (**u**), $[M-C_5H_9-88]$ (**v**) and $[M-C_5H_9-2\times88]$ (**w**) ions, respectively (Fig. 10).

Elimination of a trimethylsilyloxy radical from the molecular ion of **9a**, followed by the loss of a trimethylsilyl radical, forms two ions of m/z 367 (**x**) and 294 (**y**) (Fig. 11).

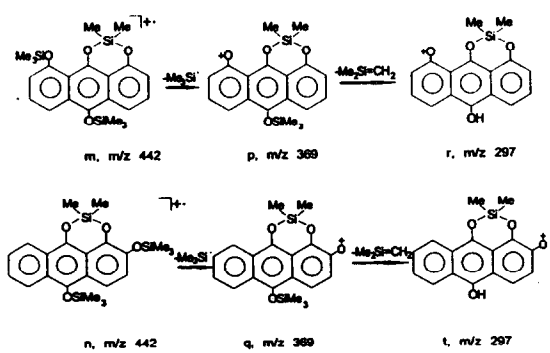


Fig. 9. Principal ions formed by the fragmentation of the reductively silylated hydroxyquinones **8** (**m**, **p**, **r**) and **10** (**n**, **q**, **t**).

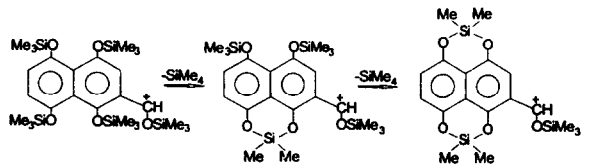


Fig. 10. Proposed fragmentation mechanism for **7a**.

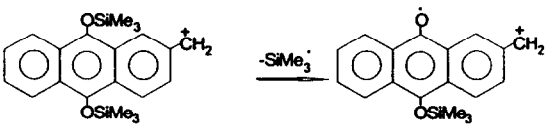


Fig. 11. Principal ions formed by the fragmentation of **9a**.

GC separation of a synthetic mixture of the hydroxyquinones studied

A typical GC separation of a synthetic mixture of all hydroxynaphtho- and hydroxyanthraquinones silyl ethers studied is presented in Fig. 12. The components were identified by co-injection with each component alone coupled by a computerized library search in the GC–MS system.

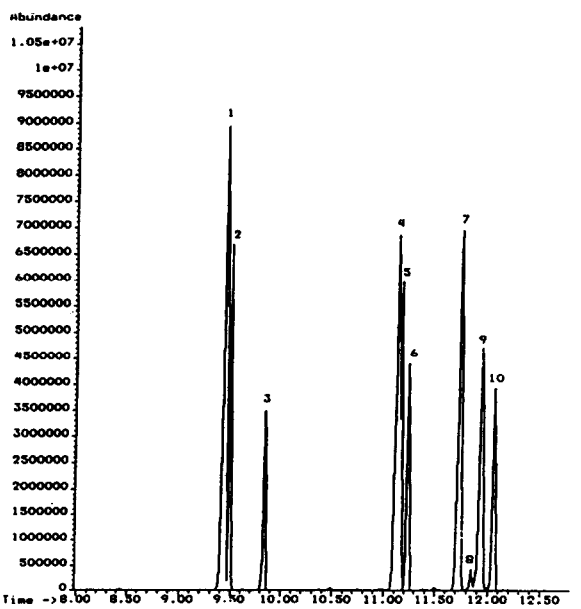


Fig. 12. Separation of a mixture of the trimethylsilylated hydroxyquinones **1–10**. For conditions, see Experimental. For peak identification, see Table II. Time in min.

In addition to the well formed peak shapes obtained and the complete separation of the mixture, especially that of the isomeric anthraquinones **5**, **6**, **8** and **10**, the retention times for the constituents of the mixture closely follow the order of the net trimethylsilylated compounds.

CONCLUSIONS

A straightforward and clear reductive trimethylsilylation reaction was applied to several hydroxyquinones, resulting in the quantitative protection of all functional groups. GC–MS proved to be a very effective method for the optimization of the silylation reaction conditions. The MS study of all silylated compounds provided some useful rules for the interpretation of the O-trimethylsilylated products. The reductive trimethylsilylation procedure is also applicable to the GC–MS separation and characterization of hydroxyquinones, even isomeric compounds, in mixtures.

REFERENCES

1 F. Arcamone, G. Cassinelli, G. Fautini, A. Grein, P. Orezzi, C. Pol and C. Spalla, *Biotechnol. Bioeng.*, 11 (1969) 1101.
2 A.J. Lin and A.C. Sartorelli, *J. Org. Chem.*, 38 (1973) 813.
3 C.A. Tsipis, E.G. Bakalbassis, V.P. Papageorgiou and M.N. Bakola-Christianopoulou, *Can. J. Chem.*, 60 (1982) 2477
4 V.P. Papageorgiou, M.N. Bakola-Christianopoulou and C.A. Tsipis, *Eur. Pat. Appl.*, No. 831116, 1983.
5 V.P. Papageorgiou, *J. Med. Plant Res.*, 38 (1980) 193.
6 H.S. Kadem (Editor), *Anthracycline Antibiotics*, Academic Press, New York, 1982.
7 D.E. Williamson and G.W. Eyerett, Jr., *J. Am. Chem. Soc.*, 97 (1975) 2397.
8 A.J. Crowe, *Chem. Ind. (London)*, (1983) 304.
9 T. Furuya, S. Shibata and H. Iizuka, *J. Chromatogr.*, 21 (1966) 116.
10 J.B. Terril and E.S. Jacobs, *J. Chromatogr. Sci.*, 8 (1970) 604.
11 G.W. Eijk and H.J. Roeijmans, *J. Chromatogr.*, 295 (1984) 497.
12 G.W. Eijk and H.J. Roeijmans, *J. Chromatogr.*, 124 (1976) 66.
13 L.M. Henriksen and H. Kjøsen, *J. Chromatogr.*, 258 (1983) 252.
14 M.N. Bakola-Christianopoulou, *J. Organomet. Chem.*, 308 (1986) C24.

15 M.N. Bakola-Christianopoulou, *J. Mol. Catal.*, 65 (1991) 307.
16 M. Donike, *J. Chromatogr.*, 42 (1969) 103.
17 S. Patai and Z. Rappoport (Editors), *The Chemistry of the Quinonoid Compounds*, Wiley, Chichester, 1988.
18 J.L. Smith, J.L. Beck and W.J.A. VandenHeuvel, *Org. Mass Spectrom.*, 5 (1971) 473.
19 W.J.A. VandenHeuvel, J.L. Smith, P. Haug and J.L. Beck, *J. Heterocycl. Chem.*, 9 (1972) 451.
20 J. Dickman, J.B. Thompson and C. Djerassi, *J. Org. Chem.*, 33 (1968) 2271.
21 E. White, V.S. Tsuboyama and J.A. McCloskey, *J. Am. Chem. Soc.*, 93 (1971) 6340.