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First direct observation of tautomerism of monohydroxynaphthazarins by IR-spectroscopy

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Dedicated to the memory of Professor Oleg B. Maximov who has been an inspiration to us over the years

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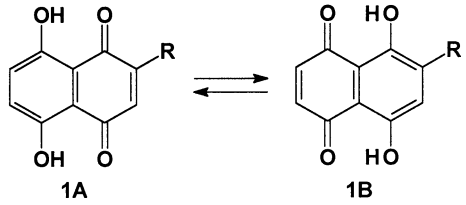
Abstract—Some substituted monohydroxylated naphthazarins (5,8-dihydroxy-1,4-naphthoquinones) were synthesized and studied by IR-spectroscopy in aprotic organic solvents at ambient temperature. Two narrow stretching mode bands in the high frequency range 3540–3410 cm^{-1} due to a β-hydroxy group were observed; it was established that this effect was caused by tautomerism. This allowed the creation of a convenient and accurate method for the measurement of a small amount of tautomer engaged in rapid exchange with the principal tautomer. Solvent and substituent effects were estimated. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Currently, there is considerable interest in the study on hydroxylated naphthazarins (5,8-dihydroxy-1,4-naphthoquinones) because of their use in the development of cardioprotective preparations[1–3] and other applications.[4,5] The known difficulties in the study of substituted hydroxynaphthazarins are related to the question of prototropic tautomerism. Indeed, ^{1}H, ^{13}C and ^{17}O NMR studies have shown that naphthazarins undergo rapid proton exchange between the α-hydroxyl and carbonyl groups giving rise to time-averaged spectra (Scheme 1).[6–8] The low activation energy for the tautomeric exchange in naphthazarins precludes the use of low temperature NMR techniques to establish the relative ratio of each component.[8,9] The presence of one or both tautomers in the equilibrium has been established on the basis of chemical proofs.[10] These proofs were supported by the trapping and isolation of derivatives where the equilibrium is 'frozen' by methylation of both α-hydroxyl groups. In this case ^{1}H NMR spectroscopy has demonstrated an ability to differentiate between tautomers. Nevertheless, as the reactivity of each tautomer can be quite different, the ratio of obtained derivatives can never be used for estimation of the composition of the tautomeric equilibrium. Moreover, both the methylating reagents and the reactive medium used could have an effect on the state of tautomeric equilibrium. The same difficulties occur in the study on acylotropic tautomerism.[11]



Scheme 1. 1 (**a**) R=OH; (**b**) R=OMe; (**c**) R=Alk; (**d**) R=STol-*p*; (**e**) R=SOTol-*p*; (**f**) R=SO_2Tol-*p*.

IR-spectroscopy is significantly more rapid in comparison with the NMR time scale and time-averaging of the spectral parameters is not generally observed because the characteristic time of the IR method is shorter than the time of the vibrational transition.[12] Based on this we have reason to expect that IR-spectroscopy could be a useful tool for studying the tautomeric equilibrium of hydroxylated naphthazarins. Recently, this expectation has been confirmed by unusual spectral manifestations of the stretching mode bands due to the β-hydroxy group (ν_{OH}) of some substituted hydroxynaphthazarins.[13]

Although hydroxynaphthazarins were expected to make a mixture of the energetically non-degenerate 1,4-dione tautomers, such behavior has not been systematically investigated through the use of IR-spectroscopy. Since a variety of mono-, di-, and trisubstituted hydroxynaphthazarins can be produced synthetically[13] and since they occur in nature,[14,15] these compounds afford an ideal subject

Keywords: naphthazarin, 5,8-dihydroxy-1,4-naphthoquinone, monohydroxylated naphthazarins; prototropic tautomerism; IR-spectroscopy of hydroxynaphthazarins in solutions.

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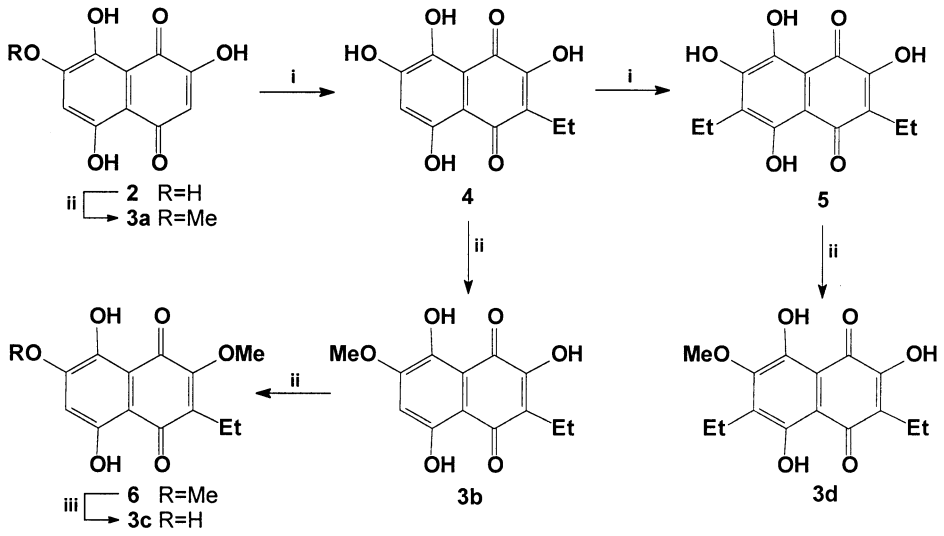


Scheme 2. (i) $(EtCOO)_2$, boiling *t*-BuOH; (ii) CH_2N_2, Et_2O; (iii) boiling conc. HCl–MeOH (1:1).

for combined theoretical and experimental studies designed to elucidate the effects of alterations of structure and polarity of solvents on tautomeric composition.

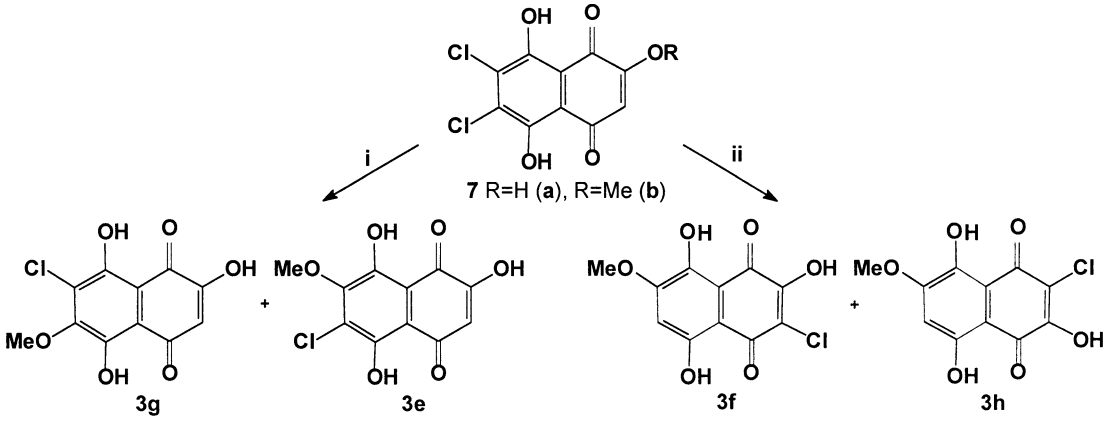
Because of the low solubility of β-hydroxysubstituted naphthazarins in non-polar or low-polarity aprotic solvents, the IR-spectra of these compounds have always been measured in KBr pellets or Nujol mulls.[14] In these cases, the spectral range (about 3500–3300 cm^{-1}) of the ν_{OH} bands is uninformative. However, the study on IR-spectra of such compounds in aprotic organic solvents have shown that the intensities and frequencies of the ν_{OH} bands could provide important information concerning tautomerism.[13]

We report here the first direct observation of the tautomerism of hydroxynaphthazarins by IR-spectroscopy in aprotic solvents.

2. Results and discussion

The tautomerism of hydroxynaphthazarins bearing both electron donating and electron withdrawing groups was studied. The compound **3a** was prepared by cautious treatment of a diluted solution of mompain (2,7-dihydroxynaphthazarin, **2**) with diazomethane in diethyl ether. The synthesis of hydroxynaphthazarins **3b–d** utilized a sequential functionalization of mompain (**2**) (Scheme 2). Mono- **4** and dialkylmompains **5** were obtained by *C*-alkylation of the starting compound **2** with propionyl peroxide in boiling *t*-BuOH. The monomethyl ethers **3b** and **d** were obtained as a result of *O*-methylation of corresponding dihydroxynaphthazarins **4** and **5** with diazomethane. Monomethyl ether **3b** was converted to its MeO-isomer **3c** via *O*-methylation with CH_2N_2 followed by mild hydrolysis of resulting dimethyl ether **6** with dilute HCl.

The chloroderivative **3e** was prepared from hydroxydichloronaphthazarin **7a** by the action of the complex reagent—MeOH/KF/Al_2O_3. The compound **7b** was converted to 2-hydroxy-3-chloronaphthazarin **3f** via nucleophilic substitution of a chlorine atom with acetoxy group by the action of a CH_3COOH/KF—reagent followed by mild alkali hydrolysis of the corresponding acetoxy derivative without its separation (Scheme 3). In both cases, the formation of corresponding 2-hydroxy-6-methoxynaphthazarins **3g** and **h** took place. The choice between **3e** and **f** isomers on the one hand, and **3g** and **h** isomers on the other was based on IR analysis of these compounds.[13] Moreover, the relative arrangement of the substituents in **3h** and **f** was



Scheme 3. (i) MeOH/KF/Al_2O_3, in closed reaction vessel, 90–95°C, 10 h; (ii) CH_3COOH/KF, reflux, 20 h; then 5% Na_2CO_3.

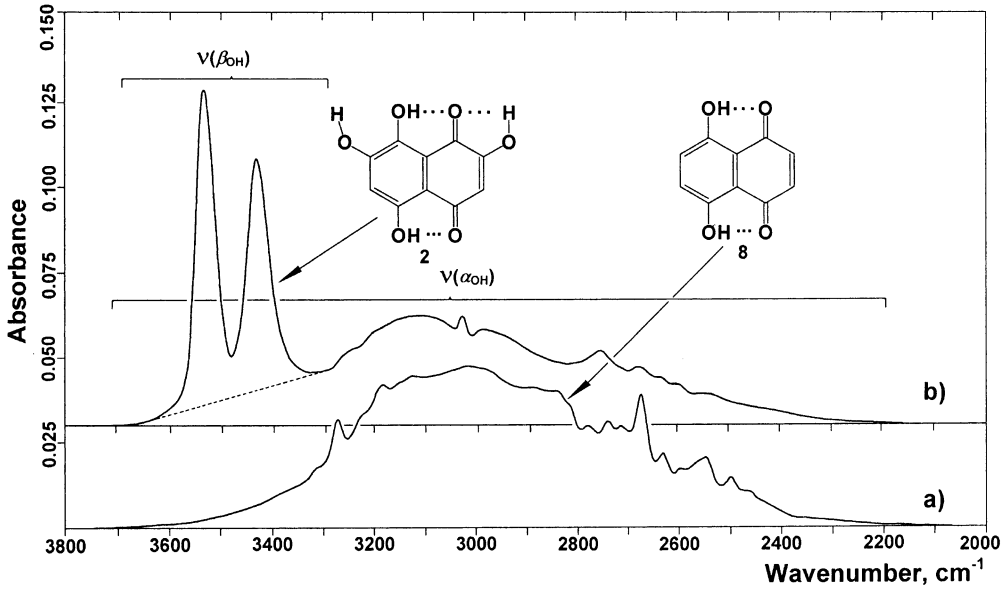


Figure 1. IR-spectra of (a) naphthazarin (**8**) and (b) mompain (**2**) in $CDCl_3$ (a region of α- and β-hydroxygroups).

confirmed by conversion of these compounds into known chlorinated 2,6- and 2,7-dihydroxynaphthazarins.[16]

Tautomerism of hydroxynaphthazarins **3a–f** was studied by IR-spectroscopy in aprotic organic solvents such as $CDCl_3$, CCl_4, and hexane at ambient temperature. It was shown previously[16] that two relatively narrow stretching mode bands were due to corresponding benzenoid and quinonoid β-hydroxy groups of 2,7-dihydroxynaphthazarin (**2**) (Fig. 1) and its C-alkyl derivatives **4** and **5** occurred at about 3520 and 3410 cm^{-1}. As in the case of naphthazarin (5,8-dihydroxy-1,4-naphthoquinone, **8**), the band caused by the stretching mode of α-hydroxy groups of mompain (**2**) and its derivatives **4**, **5**, exhibits a very wide absorption in the range 3600–2200 cm^{-1} (Fig. 1).

The ratio of the benzenoid and the quinonoid absorption (R_0) in the IR-spectra of compounds **2** and **5** may be used as a measure to determine the tautomeric composition of substituted monohydroxynaphthazarins **3a–f** in solution. As the ν_{OH} bands in the spectra of mompain (**2**) and its derivative **5** are overlapped by a low frequency wing of wide diffuse band of the stretching mode of α-hydroxy groups, their peak intensity should be specified relative to a local base line 3650–3300 cm^{-1} (dashed line in Fig. 1). The measurement of R_0 for mompain and its derivative **5** in $CDCl_3$ was estimated at 1.52 ± 0.02 (error is $\pm1.3\%$). Variation of solvents ($CDCl_3$, CCl_4 and hexane) did not affect (within $\pm1\%$) the values of R_0 as it was shown for 2,7-dihydroxy-3,6-diethylnaphthazarin (**5**). It is easy to see that if the ratio of peak intensities of ν_{OH} bands in spectra

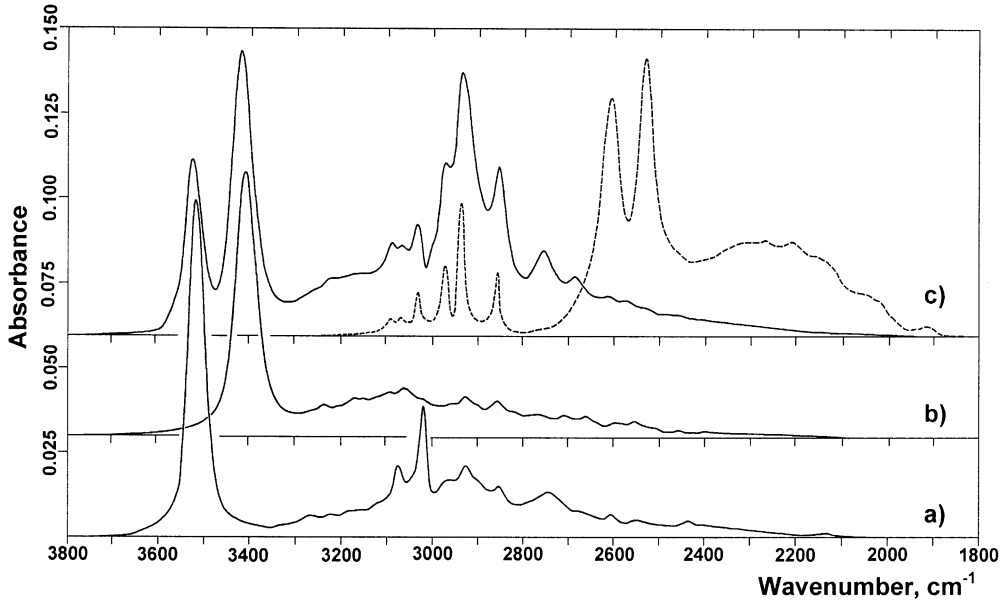
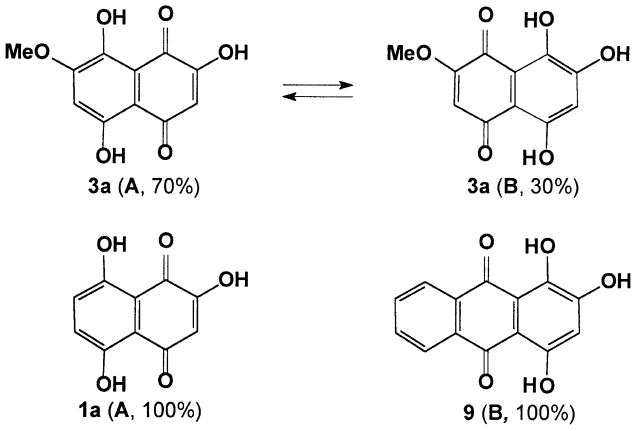


Figure 2. IR-spectra of (a) purpurin (**9**), (b) naphthopurpurin (**1a**), and (c) 2-hydroxy-7-methoxynaphthazarin (**3a**) and its derivative-d_3 (dashed line) (a region of α- and β-hydroxy groups).



Scheme 4.

Table 1. Spectral characteristics of the stretching mode bands due to a β-hydroxy group and the contents of the tautomeric forms A and B of compounds **1a**, **3a**–**f**, and **9** in $CDCl_3$

N	ν_{OH} (cm^{-1}) (**A**)	ν_{OH} (cm^{-1}) (**B**)	Form **A** (%)	Form **B** (%)
1a	3412	–	100	0
	3412[a]	–	100	0
9	–	3519	0	100
	–	3527[a]	0	100[a]
3a	3422	3526	70.0	30.0
	3420[a]	3533[a]	74.3[a]	25.7[a]
3b	3423	3525	85.7	14.3
	3420[a]	3533[a]	86.7[a]	13.3[a]
	3422[b]	3542[b]	87.3[b]	12.7[b]
3c	3414	3523	84.0	16.0
3d	3417	3524	95.6	4.4
	3414[a]	3531[a]	96.0[a]	4.0[a]
	3417[b]	3539[b]	96.0[b]	4.0[b]
3e	3418	3520	80.0	20.0
3f	3406	3514	75.8	24.2

[a] In CCl_4.
[b] In hexane.

So, both benzenoid (3526 cm^{-1}) and quinonoid (3422 cm^{-1}) ν_{OH} bands are observed in the IR-spectra of 2-hydroxy-7-methoxynaphthazarin (**3a**) in $CDCl_3$ (Fig. 2). Two ν_{OD} bands (2609 and 2533 cm^{-1}) are also observed in the IR-spectra of O-deuterated analog of **3a** with isotopic ratio ν_{OH}/ν_{OD}=1.35 (Fig. 2, dashed line). In this case, the stretching mode band of α-OD groups is shifted to about 2300 cm^{-1} (isotopic ratio $\nu_{OH}/\nu_{OD}\approx$1.30).

The ratio of benzenoid and quinonoid absorption (*r*) is equal to 0.65, and according to formula (1), the content of **3a** (**A**) in the tautomeric mixture is 70.0% (Scheme 4). On the other hand, only one ν_{OH} quinonoid (3412 cm^{-1}) or benzenoid (3519 cm^{-1}) band is observed in the IR-spectra of naphthopurpurin (**1a**) and purpurin (**9**) (Fig. 2) indicating that these compounds exist as forms **A** or **B**, respectively, and no other forms. Quantum-mechanical calculations predict the existence of compounds **1a** and **9** in forms **A** and **B**, respectively.[17,18]

The data on the tautomeric composition of studied hydroxynaphthazarins **1a**, **3a**–**f**, and purpurin (**9**) are presented in Table 1.

The study of the IR-spectra of mompain monomethyl ether (**3a**) and its derivatives demonstrates that non-polar solvents (CCl_4, hexane) shift the equilibrium to form **A** (Fig. 3). Thus, for compound **3a**, the shift was 4.3±0.1% going from $CDCl_3$ to CCl_4 (Table 1).

Thus, 2-hydroxy-7-methoxynaphthazarin (**3a**) in $CDCl_3$ was found to exist predominantly as form **A**. The introduction of electron donating ethyl group in positions 3 or 6 shifts the tautomeric equilibrium of corresponding compounds **3b** and **c** to form **A** on 16 and 14% with respect to the starting compound **3a**. When these substituents occur in positions 3 and 6, the maximal displacement of tautomeric equilibrium

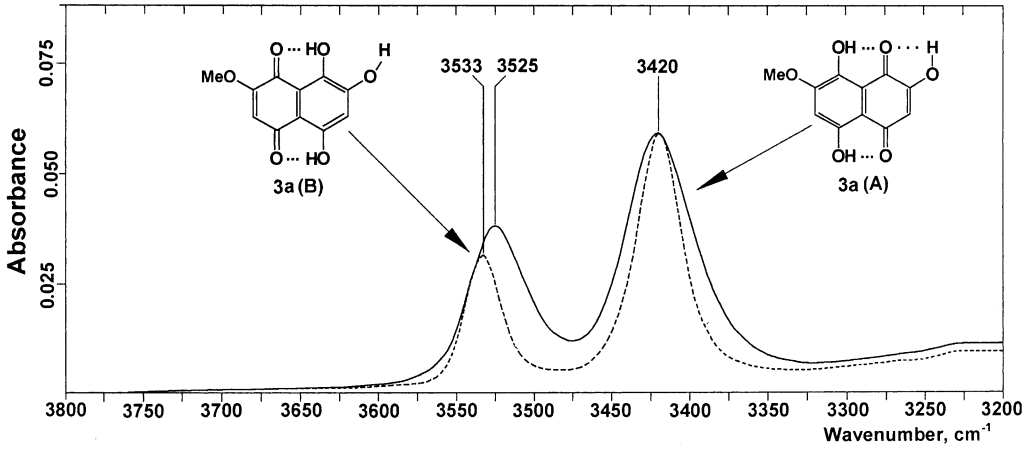


Figure 3. IR-spectra of 2-hydroxy-7-methoxynaphthazarin (**3a**) in $CDCl_3$ and CCl_4 (dashed line) (a region of β-hydroxy groups).

of hydroxynaphthazarins is *r*,[†] so the percentage of the tautomeric form **A** (Scheme 1) can be calculated as follows:

$$\mathbf{A}\ (\%) = 100/(1 + r/1.52) \quad (1)$$

[†] The base line was drawn by analogy with a measurement of R_0.

to form **A** in a solution of **3d** in $CDCl_3$ is observed (Table 1). The surprising thing is that the tautomeric equilibrium in a $CDCl_3$ solution of hydroxymethoxynaphthazarins **3e**, **f** containing the electron withdrawing chlorine atom is shifted to the same form **A** as in the case of **3b** and **c**. This is in contrast to early observations of tautomerism in

naphthazarin thioderivatives **1d–f**.[10] It is apparent that the influence of electron donating or electron withdrawing groups is insufficient for an estimation of tautomeric equilibrium. Additionally, a more accurate description of tautomeric equilibrium must take into account the probability of intramolecular hydrogen bond formation with the participation of substituents. The latter remains without comment.[10]

3. Conclusion

We have stated a convenient and accurate method based on IR spectral parameters of β-hydroxy groups to measure the quantitative composition of tautomeric equilibrium in monohydroxynaphthazarin derivatives. The proposed method demonstrates the possibility of measuring a small amount (less than 5%) of another tautomer in rapid exchange with the principal tautomer. In our opinion, it is the first example of a direct observation of both the presented tautomers at equilibrium in a room temperature solution.

4. Experimental

4.1. General comments

All melting points were determined with a Boetius apparatus and are uncorrected. The IR absorption spectra of solution of the samples in $CDCl_3$, CCl_4 and *n*-hexane at concentrations of 1×10^{-2}–5×10^{-4} M were recorded at room temperature (23±1°C) on a Bruker Vector 22 with resolution 2 cm^{-1} using matched cells (path length 0.4–2.0 mm) with CaF_2 windows supplied with polyethylene spacers. All solvents were freshly distilled and stored over molecular sieves (4 Å) prior to use. The samples were dried over P_2O_5 at room temperature, 20 h, 5 mmHg, and all operations on solutions preparation and cell refillement were performed in a dry box. Deuteration of compound **3a** was performed in dried acetone using D_2O as a deuteration reagent. The spectral parameters were measured using the OPUS/IR-2 version 3.02 software incorporated into the hardware of the instrument. ^{1}H NMR spectra were obtained on a Bruker AC-250 instrument using $CDCl_3$ as solvents unless otherwise noted and Me_4Si as an internal reference (δ=0). Coupling constants in parenthesis are reported in Hz. Mass spectra were taken on a LKB-9000S spectrometer (direct sample inlet, ionizing energy 70 eV and elevated temperature). The course of the reaction was monitored and the purity of the compounds obtained was checked by TLC (Merck Kieselgel 60F-254 plates were preliminary treated with 0.05 M tartaric acid in MeOH and dried at ~50°C for 2–3 h; a 2:1 hexane–acetone mixture was used as the eluent). Preparative TLC was performed on silica gel L (Chemapol®, Czechia), 5/40 μm. The yields were not optimized. Elemental microanalyses were performed with the Flash EA1112 CHN/MAS200 by the Elemental Analyses Services of the Far Eastern National University (FENU), Vladivostok.

The following starting compounds were prepared according to the earlier described procedures: 2,5,8-trihydroxy-1,4-naphthoquinone (**1a**),[19] 2,5,7,8-tetrahydroxy-1,4-naphthoquinone (**2**),[20] 6,7-dichloro-2,5,8-trihydroxy-1,4-naphthoquinone (**7a**),[1] 1,2,4-trihydroxy-9,10-anthraquinone (**9**).[19]

4.1.1. Radical alkylation of mompain (2). Propionyl peroxide[21] was added dropwise to a boiling solution of mompain (**2**, 110 mg, 0.5 mmol) in *tert*-BuOH (30 mL). The course of the reaction was monitored by TLC. The reaction was terminated when the degree of conversion reached ~60%. Solvent was evaporated under reduced pressure and the residue was purified by preparative TLC (hexane/acetone=2:1). After usual work-up, the main fractions were isolated:

4.1.1.1. 3,6-Diethyl-2,5,7,8-tetrahydroxy-1,4-naphthoquinone (5). 5 mg (6% with respect to consumed mompain (**2**)), R_f=0.43. Mp 185–187°C. IR ($CDCl_3$), ν (cm^{-1}): 3533 m, 3427 m (β-OH), 1630 sh. m, 1602 s (C=O), 1587 s (C=C). ^{1}H NMR: 1.19 (t, J=7.7 Hz, 6H, $2CH_3$); 2.72 (q, J=7.7 Hz, 4H, $2CH_2$); 6.73 (broad s, 2H, 2β-OH); 11.79 and 13.54 (both s, on 1H each, α-OH). EI-MS *m/z*: 278 $[M]^+$ (99), 277 $[M-1]^+$ (100), 263 (22), 262 (18), 247 (13), 235 (18). Anal. calcd for $C_{14}H_{14}O_6$: C, 60.43; H, 5.07. Found: C, 60.31; H, 5.68.

4.1.1.2. 3-Ethyl-2,5,7,8-tetrahydroxy-1,4-naphthoquinone (4). 40 mg (51% with respect to consumed mompain (**2**)), R_f=0.36. Mp 183–186°C (subl.) (lit.[22] Mp 182–188°C). IR ($CDCl_3$), ν (cm^{-1}): 3531 m, 3430 m (β-OH), 1631 m, 1603 s (C=O, C=C). ^{1}H NMR: 1.19 (t, J=7.7 Hz, 3H, CH_3); 2.68 (q, J=7.7 Hz, 2H, CH_2); 6.65 (s, 1H, H(6)), 6.90 (broad s, 1H, β-OH); 11.70 and 13.11 (both s, on 1H each, α-OH). EI-MS *m/z*: 250 $[M]^+$ (100), 249 $[M-1]^+$ (48), 235 (8), 207 (29), 206 (13).

Fraction with R_f=0.29 was the starting mompain (**2**), 41 mg (37%).

4.1.2. Methylation of naphthazarin derivatives 2, 4, 5, and 7a by CH_2N_2. The compounds **3a**, **b**, **d**, and **7b** were obtained by reaction of corresponding naphthazarins **2**, **4**, **5** and **7a** (0.3 mmol) in Et_2O with solution of CH_2N_2 in Et_2O[23] monitored by TLC. The reaction mixture was concentrated in vacuo to give a residue which was purified by preparative TLC (hexane/acetone=3:1).

4.1.2.1. 2,5,8-Trihydroxy-7-methoxy-1,4-naphthoquinone (3a). From **2**, 25 mg (35%), R_f=0.28. Mp 225–227°C (lit.[24] Mp 240–241°C). IR ($CDCl_3$), ν (cm^{-1}): 3525 m, 3422 m (β-OH), 1661 m, 1629 m, 1606 s (C=O), 1585 sh. w (C=C). ^{1}H NMR: 3.97 (s, 3H, OCH_3); 6.48 and 6.53 (both s, on 1H each, H(6), H(3)); 12.08 and 13.11 (both s, on 1H each, α-OH). EI-MS *m/z*: 236 $[M]^+$ (100), 223 (9), 218 (22), 208 (13), 206 (17), 205 (11), 193 (15), 190 (14). In addition to product **3a**, 5,8-dihydroxy-2,7-dimethoxy-1,4-naphthoquinone was isolated from the reaction mixture in a yield 46 mg (61%), R_f=0.34. Mp 270–272°C (lit.[24] Mp 273–275°C). ^{1}H NMR: 3.96 (s, 6H, $2OCH_3$); 6.40 (s, 2H, H(6), H(3)); 12.73 and 13.15 (both s, on 1H each, α-OH).

4.1.2.2. 2,5,8-Trihydroxy-7-methoxy-3-ethyl-1,4-naphthoquinone (crystazarin, 3b). From **4**, 54 mg (68%), R_f=0.41. Mp 230–232°C (lit.[24] Mp 230–232°C,

lit.[25] Mp 154–157°C). IR ($CDCl_3$), ν (cm^{-1}): 3526 m, 3424 m (β-OH), 1654 w, 1629 m, 1599 s (C═O), 1577 sh. m (C═C). ^1H NMR: 1.16 (t, J=7.6 Hz, 3H, CH_3); 2.64 (q, J=7.6 Hz, 2H, CH_2); 3.96 (s, 3H, OCH_3); 6.57 (s, 1H, H(6)); 7.13 (broad s, 1H, β-OH); 12.04 and 13.34 (both s, on 1H each, α-OH). ^1H NMR (DMSO-d_6): 1.05 (t, J=7.6 Hz, 3H, CH_3); 2.50 (q, J=7.6 Hz, 2H, CH_2); 3.91 (s, 3H, OCH_3); 6.76 (s, 1H, H(6)); 11.11 (broad s, 1H, β-OH); 12.27 and 13.50 (both s, on 1H each, α-OH). EI-MS m/z: 264 $[M]^+$ (100). In addition to product **3b**, 3-ethyl-5,8-dihydroxy-2,7-dimethoxy-1,4-naphthoquinone (**6**) was isolated from the reaction mixture in a yield 27 mg (32%), R_f=0.45. Mp 146–149°C (lit.[22,24,26] Mp 145–147°C). ^1H NMR: 1.16 (t, J=7.6 Hz, 3H, CH_3); 2.73 (q, J=7.6 Hz, 2H, CH_2); 3.94 and 4.06 (both s, on 3H each, OCH_3); 6.26 (s, 1H, H(6)); 12.80 and 13.31 (both s, on 1H each, α-OH). EI-MS m/z: 278 $[M]^+$ (100).

4.1.2.3. 3,6-Diethyl-2,5,8-trihydroxy-7-methoxy-1,4-naphthoquinone (3d). From **5**, 32 mg (36%), R_f=0.56. Mp 115–117°C. IR ($CDCl_3$), ν (cm^{-1}): 3519 w, 3417 m (β-OH), 1623 m, 1600 s (C═O), 1575 sh. m (C═C). ^1H NMR: 1.15 and 1.18 (both t, on 3H each, J=7.6 Hz, CH_3); 2.62 and 2.76 (both q, on 2H each, J=7.6 Hz, CH_2); 4.03 (s, 3H, OCH_3); 12.01 and 13.54 (both s, on 1H each, α-OH). EI-MS m/z: 292 $[M]^+$ (17), 291 $[M-1]^+$ (16), 258 (37), 256 (100), 255 (15). Anal. calcd for $C_{15}H_{16}O_6$: C, 61.64; H, 5.52. Found: C, 61.91; H, 5.68.

4.1.2.4. 6,7-Dichloro-5,8-dihydroxy-2-methoxy-1,4-naphthoquinone (7b). This compound was prepared from **7a** in quantitative yield. Mp 216–218°C. ^1H NMR: 3.98 (s, 3H, OCH_3); 6.29 (s, 1H, H(3)); 12.77 and 13.28 (both s, on 1H each, α-OH). EI-MS m/z: 288/290/292 $[M]^+$ (100), 287/289/291 $[M-1]^+$ (88), 273/275/277 (23), 272/274/276 (44), 270/272/274 (75), 269/271/273 (59), 258 (10), 256 (10), 223 (14), 222 (10). Anal. calcd for $C_{11}H_6O_5Cl_2$: C, 45.70; H, 2.09. Found: C, 45.92; H, 2.00.

4.1.3. Partial acid hydrolysis of substituted 2,7-dimethoxynaphthazarin 6. To a boiling solution of 6-ethyl-5,8-dihydroxy-2,7-dimethoxy-1,4-naphthoquinone (**6**, 53 mg, 0.2 mmol) in MeOH (30 mL) was added dropwise over 3 min HCl (30 mL), and the mixture was heated at reflux for 60 min monitored by TLC. After cooling, the mixture was diluted by H_2O (50 mL), then extracted three times with diethyl ether. The organic extract was washed with brine, and dried over anhydrous Na_2SO_4 and concentrated. Preparative TLC (hexane/acetone=3:1) yielded 6-ethyl-2,5,8-trihydroxy-7-methoxy-1,4-naphthoquinone (**3c**), 22 mg (42%), R_f=0.42. Mp 160–166°C. IR ($CDCl_3$), ν (cm^{-1}): 3523 w, 3414 m (β-OH), 1662 m, 1620 s, 1614 s (C═O), 1588 s, 1575 sh. s (C═C). ^1H NMR: 1.19 (s, J=7.5 Hz, 3H, CH_3); 2.75 (q, J=7.5 Hz, 2H, CH_2); 4.04 (s, 3H, OCH_3); 6.36 (s, 1H, H(3)); 12.05 and 13.35 (both s, on 1H each, α-OH). EI-MS m/z: 264 $[M]^+$ (100), 250 (17), 249 (45). Anal. calcd for $C_{13}H_{12}O_6$: C, 58.09; H, 4.58. Found: C, 58.13; H, 4.59.

4.1.4. 6-Chloro-2,5,8-trihydroxy-7-methoxy-1,4-naphthoquinone (3e). A mixture of well-dried 6,7-dichloro-2-hydroxynaphthazarin (**7a**) (65 mg, 0.23 mmol), anhydrous KF (100 mg, 1.72 mmol), activated neutral alumina (Aldrich, ~150 mesh, for chromatography) (640 mg), absolute MeOH (10 mL),[‡] and ethylene glycol dimethyl ether (2 mL) was stirred in a closed reaction vessel at 90–95°C for 10 h. After cooling, the absorbent was separated by filtration, washed successively with 5% HCl (0.5 mL) and acetone (3 mL). The combined acidic filtrate was concentrated in vacuo, and the residue was treated with water (3 mL) and then Et_2O. The organic layer was washed with water and brine, dried Na_2SO_4, filtered and concentrated. Preparative TLC (hexane/acetone=3:1) afforded the product **3e**, 27 mg (42%), R_f=0.32. Mp 234–235°C. IR ($CDCl_3$), ν (cm^{-1}): 3520 m, 3418 m (β-OH), 1663 w, 1620 m, 1606 s (C═O), 1575 m (C═C). ^1H NMR: 4.15 (s, 3H, OCH_3); 6.45 (s, 1H, H(3)); 11.97 and 13.37 (both s, on 1H each, α-OH). EI-MS m/z: 270/272 $[M]^+$ (100), 269/271 $[M-1]^+$ (36). Anal. calcd for $C_{11}H_7O_6Cl$: C, 48.82; H, 2.61. Found: C, 48.23; H, 2.60.

In addition to product **3e**, 7-chloro-2,5,8-trihydroxy-6-methoxy-1,4-naphthoquinone (**3g**) was isolated from the reaction mixture in a yield of 13 mg (20%), R_f=0.41. Mp 200–204°C. IR ($CDCl_3$), ν (cm^{-1}): 3515 m, 3409 m (β-OH), 1661 w, 1624 sh. m, 1607 s (C═O), 1580 m, 1567 m (C═C). ^1H NMR: 4.26 (s, 3H, OCH_3); 6.44 (s, 1H, H(3)); 12.32 and 13.24 (both s, on 1H each, α-OH). EI-MS m/z: 270/272 $[M]^+$ (100), 269/271 $[M-1]^+$ (20), 252/254 (39), 241/243 (35), 235 (22), 224/226 (22). Anal. calcd for $C_{11}H_7ClO_6$: C, 48.82; H, 2.61. Found: C, 48.68; H, 2.69.

4.1.5. 3-Chloro-2,5,8-trihydroxy-7-methoxy-1,4-naphthoquinone (3f). A mixture of well-dried 6,7-dichloro-2-methoxynaphthazarin (**7b**) (70 mg, 0.24 mmol), anhydrous KF (150 mg, 2.6 mmol), and glacial acetic acid (15 mL) was refluxed for 20 h. After cooling, the reaction mixture was concentrated in vacuo. The residue was treated with 5% Na_2CO_3 to pH 8–9 for about 15 min, monitoring by TLC. The mixture was acidified by 10% HCl to pH 4–5 and extracted with Et_2O. The organic layer was washed with water and brine, dried Na_2SO_4 and concentrated. Preparative TLC using $CHCl_3$, yielded **3f**, 35 mg (53%), R_f=0.28. Mp 207–209°C. IR ($CDCl_3$), ν (cm^{-1}): 3514 m, 3406 m (β-OH), 1670 w, 1633 m, 1606 s (C═O), 1585 sh. m (C═C). ^1H NMR: 3.99 (s, 3H, OCH_3); 6.62 (s, 1H, H(6)); 12.02 and 12.99 (both s, on 1H each, α-OH). EI-MS m/z: 270/272 $[M]^+$ (100), 269/271 $[M-1]^+$ (96), 256/258 (61), 191 (27). Anal. calcd for $C_{11}H_7O_6Cl$: C, 48.82; H, 2.61. Found: C, 48.68; H, 2.98.

In addition to product **3f**, 3-chloro-2,5,8-trihydroxy-6-methoxy-1,4-naphthoquinone (**3h**) was isolated from the reaction mixture in a yield of 28 mg (42%), R_f=0.29. Mp >250°C (subl.). IR ($CDCl_3$), ν (cm^{-1}): 3515 m, 3376 m (β-OH), 1678 w, 1634 m, 1603 s (C═O), 1580 sh. m (C═C). ^1H NMR: 4.02 (s, 3H, OCH_3); 6.56 (s, 1H, H(7)); 12.04 and 13.19 (both s, on 1H each, α-OH). EI-MS m/z: 270/272 $[M]^+$ (100), 269/271 $[M-1]^+$ (92), 252/254 (42), 241/243 (22), 240/242 (19), 236/238 (19), 223 (18). Anal. calcd for $C_{11}H_7O_6Cl$: C, 48.82; H, 2.61. Found: C, 48.90; H, 2.96.

[‡] Reagents were prepared according to the early described procedure.[27]

4.1.6. Hydrolysis of compounds 3f and 3h. Monomethyl ether **3f** or **h** (25 mg, 0.09 mmol) in concentrated HBr (2 mL) was refluxed for 30 min. The reaction mixture was diluted with H_2O (5 mL) and extracted with ethyl acetate (3×5 mL). The extract was concentrated and isolated by preparative TLC (hexane/acetone=3:1).

4.1.6.1. 3-Chloro-2,5,7,8-tetrahydroxy-1,4-naphthoquinone. This compound was prepared by the above mentioned procedure from 3-chloro-2,5,8-trihydroxy-7-methoxy-1,4-naphthoquinone (**3f**) in quantitative yield, R_f=0.33. Mp 227–230°C (with decomp.). IR ($CDCl_3$), ν (cm^{-1}): 3523 m, 3413 m (β-OH), 1632 m, 1609 s (C=O), 1595 sh. m (C=C). ^1H NMR (acetone-d_6): 6.62 (s, 1H, H(6)); 10.52 (broad s, 1H, β-OH); 12.31 and 13.03 (both s, on 1H each, α-OH). EI-MS *m/z* (%): 256/258 $[M]^+$ (100), 228/230 (42), 200 (5), 193 (12), 186 (17). Anal. calcd for $C_{10}H_5O_6Cl$: C, 46.81; H, 1.96. Found: C, 46.74; H, 2.01.

4.1.6.2. 3-Chloro-2,5,6,8-tetrahydroxy-1,4-naphthoquinone. This compound was prepared by the above mentioned procedure from 3-chloro-2,5,8-trihydroxy-6-methoxy-1,4-naphthoquinone (**3h**) in quantitative yield, R_f=0.28. Mp 257–261°C. IR ($CDCl_3$), ν (cm^{-1}): 3508 m, 3373 m (β-OH), 1610 s (C=O), 1594 sh. s (C=C). ^1H NMR (acetone-d_6): 6.65 (s, 1H, H(6)); 10.16 (broad s, 1H, β-OH); 12.08 and 13.00 (both s, on 1H each, α-OH). EI-MS *m/z* (%): 257/259 (40), 256/258 $[M]^+$ (100), 255/257 (11), 228/230 (13), 224 (9), 223 (20), 222 (75). Anal. calcd for $C_{10}H_5O_6Cl$: C, 46.81; H, 1.96. Found: C, 46.73; H, 2.00.

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