# $^{15}$N NMR STUDY OF AMINO–IMINO TAUTOMERISM IN DERIVATIVES OF 1,4-BIS(SUBSTITUTED AMINO)-9,10-ANTHRAQUINONES AND 1,4-BIS(SUBSTITUTED AMINO)-2,3-DIHYDRO-9,10--ANTHRAQUINONES

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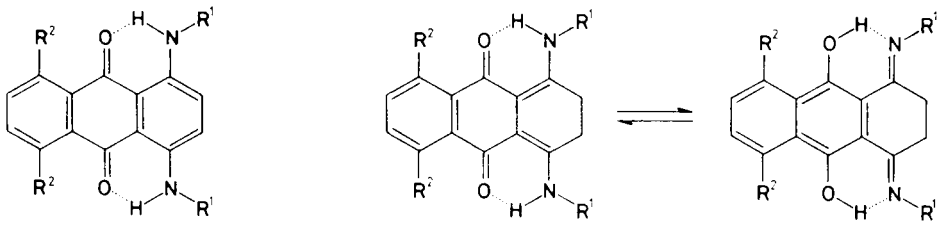
The $^{15}$N chemical shifts and $^{1}J(^{15}N, H)$ coupling constants of 1,4-bis(substituted amino)-9,10--anthraquinones and 1,4-bis(substituted amino)-2,3-dihydro-9,10-anthraquinones indicate that these derivatives exist as true aminoderivatives except for 1,4-bis(phenylamino)-2,3-dihydro--9,10-anthraquinone which forms a tautomeric mixture of the amino and imino forms in deuteriochloroform and hexadeuteriodimethyl sulphoxide.

Recently a synthesis has been described[1,2] of substituted 1,4-bis(alkylamino)-9,10--anthraquinones (*I*) with antitumour effects. The compounds *I* can be prepared by oxidation of their leucoforms *II* (Scheme 1). Structure of the leucoforms was investigated. Bloom and Hutton[3] suggested that the leucoform of 1,4-bis(benzylamino)--9,10-anthraquinone corresponds to 1,4-bis(benzylamino)-2,3-dihydro-9,10-anthraquinone in accordance with the sharp methylene group singlet found in the $^{1}$H NMR spectrum at 2·68 ppm. Moreover, the loss of aromaticity in this type of compounds was proved by the Japanese authors[4] who used the $^{13}$C NMR spectra to appreciate



*I* *II A* *II B*

In formulae *I*, *II* : *a*, $R^1$ = H ; $R^2$ = H ; *b*, $R^1$ = $CH_2CH_2NHCH_2CH_2OH$ ; $R^2$ = H ; *c*, $R^1$ = $CH_2CH_2OH$ ; $R^2$ = OH ; *d*, $R^1$ = $CH_2CH_2N(CH_2CH_3)_2$ ; $R^2$ = OH ; *e*, $R^1$ = $CH_2CH_2NHCH_2CH_2OH$ ; $R^2$ = OH , *f*, $R^1$ = $C_6H_5$ ; $R^2$ = H ; *g*, R = $C_6H_5$ ; R = OH

SCHEME 1

the structure of leucoforms of 9,10-anthraquinones. However, compounds *II* (and similarly compounds *I*, too) can exist in two tautomeric forms *IIA* and *IIB* (Scheme 1), *ie.* either as amino compounds or as imino compounds or as their mixture. The $^{15}$N NMR spectroscopy appears to be the most suitable method for appreciation of the amino–imino tautomerism. The aim of this work was to measure the $^{15}$N chemical shifts and $^{1}J(^{15}N, H)$ coupling constants of the nitrogen atoms directly involved in the possible tautomeric system of compounds *Ia*–*Ig* and *IIa*–*IIg* and to appreciate, with the help of these data, the existence of the amino–imino tautomerism.

## EXPERIMENTAL

The $^{15}$N and $^{1}$H NMR spectra were measured by means of a JNM-FX 100 (JEOL) apparatus at 10·095 and 99·602 MHz, resp., in the pulse mode. The samples for the measurements were dissolved in $C^2HCl_3$ or [$^2H_6$]-dimethyl sulphoxide. Typical parameters of the $^{15}$N NMR measurements: 45° pulse width, 8 K, 5 000 Hz spectral width, 5 s pulse repetition, the proton noise decoupling and the gated decoupling, resp. The $^{15}$N chemical shifts are related to external neat nitromethane (25% $^{15}$N; $\delta(^{15}N) = 0{\cdot}0$). The $^{1}$H NMR spectra were measured in the standard way with digital resolution of 0·2 Hz/point. The temperatures given in Tables I and II correspond to those of the gaseous cooling/heating medium measured with a thermocouple in the probe with an accuracy of ±1 K.

Compounds *Ia* and *IIa* were commercial products (Východočeské chemické závody, Pardubice). Compound *IIb*–*IIe* (Scheme 1) were prepared according to refs[1,2] by reaction of leucoforms of 1,4-dihydroxy-9,10-anthraquinone (existing as 2,3-dihydro-9,10-dihydroxyanthraquinones[4]) or 1,4,5,8-tetrahydroxy-9,10-anthraquinone with the respective aliphatic amines in inert atmosphere. Compounds *Ib*–*Ie* were prepared by oxidation[1,2] of the respective compounds *II*.

Preparation of 1,4-Bis(phenylamino)-2,3-dihydro-9,10-anthraquinone (*IIf*)

A mixture of 1 g (4 mmol) leucoform of 1,4-dihydroxy-9,10-anthraquinone, 1 g boric acid, 3 ml acetic acid, and 3 ml aniline was stirred in an inert atmosphere at 125°C 2 h, then it was cooled to 80°C and diluted with 10 ml toluene. The precipitate of boroacetate complex of compound *IIf* was collected by suction, washed with toluene and petroleum ether, and dried. The decomposition of the boroacetate complex was carried out in an inert atmosphere by 2 h boiling in 30 ml aqueous acetone (1 : 1) to give 0·9 g metallic-brown crystalline solid which decomposes at 207 to 225°C. Purity and uniformity of the product were checked by TLC (Silufol, hexane–ethyl acetate 3 : 1; $R_F = 0{\cdot}71$).

[$^{15}N_2$]-1,4-*Bis(phenylamino)*-2,3-*dihydro*-9,10-*anthraquinone* was prepared similarly from $^{15}$N-aniline (95% $^{15}$N, Isocommerz, Berlin).

Preparation of 1,4-Bis(phenylamino)-9,10-anthraquinone (*If*)

A mixture of 2·5 ml nitrobenzene and 0·45 g (1·1 mmol) compound *IIf* was heated at 125°C 1·5 h. After cooling, the product was precipitated by addition of 5 ml hexane. Yield 0·3 g (0·8 mmol) dark blue crystalline solid, m.p. 219·5–222°C (toluene) (ref.[5] gives m.p. 218°C); $R_F = 0{\cdot}94$ in the same solvent as for *IIf*.

[$^{15}N_2$]-1,4-*Bis(phenylamino)*-9,10-*anthraquinone* was prepared similarly from [$^{15}N_2$]-*IIf*.

Preparation of 1,4-Bis(phenylamino)-5,8-dihydroxy-9,10-anthraquinone (*Ig*)

The boroacetate complex was prepared by introducing 2 g (7·3 mmol) leucoform of 1,4,5,8-tetrahydroxy-9,10-anthraquinone into a solution of 2 g boric acid in 10 ml acetanhydride at 100°C in an inert atmosphere. After 30 min, the reaction mixture was cooled, and the separated complex was collected by suction (2·3 g, 4·3 mmol), whereupon it was heated with 20 ml aniline at 130°C 1·5 h. The reaction mixture was cooled to 80°C, and the product was precipitated by addition of 20 ml ethanol. The precipitate was extracted with toluene, and the extract was concentrated to give 0·8 g blue-black compound *Ig*, m.p. 266–268°C (toluene) (ref.[6] gives m.p. 258–260°C). Purity and uniformity of the product were checked by TLC (Silufol, hexane–acetone 3 : 1; $R_F = 0{\cdot}91$).

[$^{15}N_2$]-1,4-*Bis(phenylamino)*-5,8-*dihydroxy*-9,10-*anthraquinone* was prepared similarly from 0·9 boroacetate complex. 0·7 g $^{15}$N-aniline (95% $^{15}$N), and 1·4 g aniline. Yield 0·6 g [$^{15}N_2$]-*Ig*.

Preparation of 1,4-Bis(phenylamino)-2,3-dihydro-5,8-dihydroxy-9,10-anthraquinone (*IIg*)

0·2 g 1,4-bis(phenylamino)-5,8-dihydroxy-9,10-anthraquinone wetted with 1 ml ethanol was introduced into a solution of 0·8 g sodium dithionite and 1 ml 30% NaOH in 10 ml water at 80°C in an inert atmosphere. The reaction mixture was heated at 100°C 30 min. After cooling, 0·15 g metallic-brown crystals was collected by suction (the decomposition temperature 254 to 260°C). Purity of the product was checked by TLC (Silufol, hexane–ethyl acetate 3 : 1; $R_F = 0{\cdot}25$). For $C_{28}H_{20}N_2O_4$ (424·4) calculated: 6·60% N; found: 6·98% N.

[$^{15}N_2$]-1,4-*Bis(phenylamino)*-2,3-*dihydro*-5,8-*dihydroxy*-9,10-*anthraquinone* was prepared similarly from [$^{15}N_2$]-1,4-bis(phenylamino)-5,8-dihydroxy-9,10-anthraquinone.

## RESULTS AND DISCUSSION

The $\delta(^{15}N)$ chemical shifts (Tables I and II) of the 1,4-bis(substituted amino)-9,10-anthraquinones *I* lie within a narrow interval from −272·7 to −297·6. Both the

TABLE I

$^{15}$N Chemical shifts in compounds *Ia–Ie* and *IIa–IIe* in deuteriochloroform and hexadeuteriodimethyl sulphoxide at 300 K

| Compound | Solvent | $\delta(^{15}N)^a$ | Compound | Solvent | $\delta(^{15}N)^a$ |
|---|---|---|---|---|---|
| *Ia* | [b] | −297·6 | *IIb* | [b] | −246·0 |
| *Ib* | [b] | −291·8 | *IIc*[f] | [b] | −244·5 |
| *Id*[c] | [d] | −287·9 | *IIc*[g] | [b] | −244·6 |
| *Ie*[e] | [d] | −287·9 | *IId*[h] | [d] | −249·7 |
| *IIa* | [b] | −264·1 | *IIe*[i] | [b] | −249·7 |

[a] ±0·2 ppm; negative values denote upfield shifts; [b] [$^2H_6$]-dimethyl sulphoxide; [c] $^1J(^{15}N, H) = 92$ Hz; [d] $C^2HCl_3$; [e] $^1J(^{15}H, N) = 91{\cdot}8$ Hz; [f] $^1J(^{15}N, H) = 88{\cdot}4$ Hz; [g] 370 K; [h] $^1J(^{15}N, H) = 90{\cdot}8$ Hz; [i] $^1J(^{15}N, H) = 87{\cdot}9$ Hz.

values $\delta(^{15}N)$ and $^1J(^{15}N, H)$ (from 90·3 to 92 Hz), as well as their temperature independence indicate that the compounds *I* exist in amino form, *i.e.* as the 1,4-bis-(substituted amino) derivatives[7,8]. In the leucoforms of compounds *IIa–IIe*, we observed a downfield shift of $\delta(^{15}N)$ by 31·7 to 45·8 ppm as compared with $\delta(^{15}N)$ of compounds *Ia–Ie*. The $^{15}N$ chemical shifts are very similar in deuteriochloroform and hexadeuteriodimethyl sulphoxide (Table I), and are temperature-independent for *IIc*. These facts and the value of $^1J(^{15}N, H) = 90·8$ Hz in compound *IId* (as well as the values of $\delta(^{15}N)$ and $^1J(^{15}N, H)$ of compound *IIg*, see below) indicate that the compounds *IIa–IIe* are (within experimental error) also aminoderivatives *IIA* (Scheme 1).

The situation, however, is different in the case of compound *IIf*. We have found that, both in deuteriochloroform and in hexadeuteriodimethyl sulphoxide, the values of $\delta(^{15}N)$ and $^1J(^{15}N, H)$, measured from the $^1H$ NMR spectrum of the

TABLE II

Temperature dependence of $^{15}N$ chemical shifts and $^1J(^{15}N, H)$ coupling constants in compounds *If, Ig, IIf, IIg* in deuteriochloroform and hexadeuteriodimethyl sulphoxide

| Compound | Solvent | Temperature, K | $\delta(^{15}N)^a$ | $^1J(^{15}N, H)^b$ | %[c] |
|---|---|---|---|---|---|
| *If* | [d] | 300 | −278·3 | 90·3 | [f] |
| *If* | [d] | 330 | −278·3 | 90·3 | [f] |
| *If* | [d] | 370 | −278·6 | 90·3 | [f] |
| *If* | [e] | 240 | −277·1 | 90·8 | [f] |
| *If* | [e] | 270 | −277·9 | 90·8 | [f] |
| *If* | [e] | 300 | −278·4 | 90·8 | [f] |
| *IIf* | [d] | 300 | −200·2 | 60·1 | 68·3 |
| *IIf* | [d] | 330 | −195·8 | 57·1 | 64·9 |
| *IIf* | [d] | 370 | −189·1 | 54·2 | 61·6 |
| *IIf* | [e] | 240 | −227·8 | 78·3 | 89·0 |
| *IIf* | [e] | 270 | −218·5 | 72·3 | 82·2 |
| *IIf* | [e] | 300 | −210·5 | 65·9 | 74·9 |
| *IIf* | [e] | 330 | −202·8 | 61·0 | 69·3 |
| *Ig* | [d] | 300 | −272·7 | 91·3 | [f] |
| *Ig* | [d] | 370 | −272·9 | 91·3 | [f] |
| *Ig* | [e] | 300 | −274·5 | 91·3 | [f] |
| *Ig* | [e] | 330 | −275·2 | 91·3 | [f] |
| *IIg* | [e] | 300 | −242·8 | 87·8 | [f] |
| *IIg* | [e] | 330 | — | 87·6 | [f] |

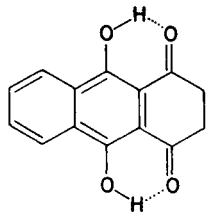
[a] ±0·2 ppm; negative values denote upfield shifts; [b] ±0·2 Hz; [c] percentage of aminoform; [d] [$^2H_6$]-dimethyl sulphoxide; [e] $C^2HCl_3$; [f] about 100%.

$^{15}$N-enriched compound (95% $^{15}$N), depend strongly on temperature (Table II). This temperature dependence is ascribed to the existence of the tautomeric equilibrium between the amino- (*IIA*) and iminoforms (*IIB*) (Scheme 1). The amino–imino equilibrium in compound *IIf* is much too rapid for the NMR time scale, hence the NMR spectra of individual tautomers cannot be observed. The content of aminoform in compound *IIf* was calculated from $^1J(^{15}\mathrm{N, H})_{exp}$ (ref.[9]) according to Eq. (*1*),

$$\% \text{ aminoform} = 100 \,.\, {}^1J(^{15}\mathrm{N, H})_{exp}/{}^1J(^{15}\mathrm{N, H}) \,, \qquad (1)$$

where $^1J(^{15}\mathrm{N, H}) = 88$ Hz (see $^1J(^{15}\mathrm{N, H})$ in *IIg*). The content of aminoform decreases with increasing temperature (Table II). From the results given it can also be seen that with decreasing value of the $^1J(^{15}\mathrm{N, H})$ the $\delta(^{15}\mathrm{N})$ value of the corresponding nitrogen exhibits the well-known downfield trend[10,11].

In order to support our suggestion, we measured the $^{13}$C chemical shifts of C=O group of compound *IIf* at different temperatures: hexadeuteriodimethyl sulphoxide: $\delta(\mathrm{CO}) = 167{\cdot}1$ (300 K); 165·5 (370 K), deuteriochloroform: $\delta(\mathrm{CO}) = 172{\cdot}2$ (240 K); 169·1 (330 K). For comparison: $\delta(^{13}\mathrm{CO})$ of 9,10-anthraquinone is 182·5 (ref.[12]) and $\delta(^{13}\mathrm{COH})$ of compound *III* is 154·9 (ref.[4]).



*III*

The upfield shift of $\delta(^{13}\mathrm{CO})$ with increasing temperature corresponds to increased significance of the *IIB* form (Scheme 1), hence the result agrees with the data obtained by calculation from Eq. (*1*) (Table II).

In order to appreciate the influence of 5,8-hydroxy groups on the amino–imino tautomerism, we prepared the compounds *Ig* and *IIg*. According to expectations, the compound *Ig* exists as the true amino compound both in deuteriochloroform and in hexadeuteriodimethyl sulphoxide. The 5,8-hydroxy groups, however, cause the compound *IIg* (in contrast to *IIf*) to exist only in the aminoform (within experimental error). The $\delta(^{15}\mathrm{N})$ value is comparable with those in *IIb*–*IIe* and differs distinctly from $\delta(^{15}\mathrm{N})$ in compound *IIf*. The $^1J(^{15}\mathrm{N, H})$ value in compound *IIg* is temperature-independent and equal to that of compound *IIe*.

The compound *IIg* is inclined to oxidation, hence the measurement of $\delta(^{15}\mathrm{N})$ and $^1J(^{15}\mathrm{N, H})$ in deuteriochloroform was carried out in a sealed NMR test tube

under nitrogen atmosphere. The compound *IIg* is not sufficiently soluble in hexadeuteriodimethyl sulphoxide, hence the measurement could not be carried out in this solvent.

The $^{15}$N-labelled compounds enable (with respect to splitting of the NH signal of $^{1}$H NMR spectrum into a doublet by the effect of the $^{15}$N isotope ($I = 1/2$)) an unambiguous assignment of the $^{1}$H chemical shifts of the two types of acidic protons in compounds *Ig* and *IIg*. In compound *Ig* it is $\delta(\mathrm{NH}) = 11{\cdot}88$ ($\mathrm{C^2HCl_3}$, 300 K) and $\delta(\mathrm{OH}) = 13{\cdot}23$, whereas for *IIg* $\delta(\mathrm{NH}) = 14{\cdot}21$ and $\delta(\mathrm{OH}) = 13{\cdot}59$.

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