

# The influence of caffeine on the coupling reaction between $\beta$ -naphthol and p-diazobenzenesulphonic acid

BY

J. TH. G. OVERBEEK, C. L. J. VINK and H. DEENSTRA

(van 't Hoff-Laboratory and Clinic for Internal Medicine  
University of Utrecht, Netherlands)



---

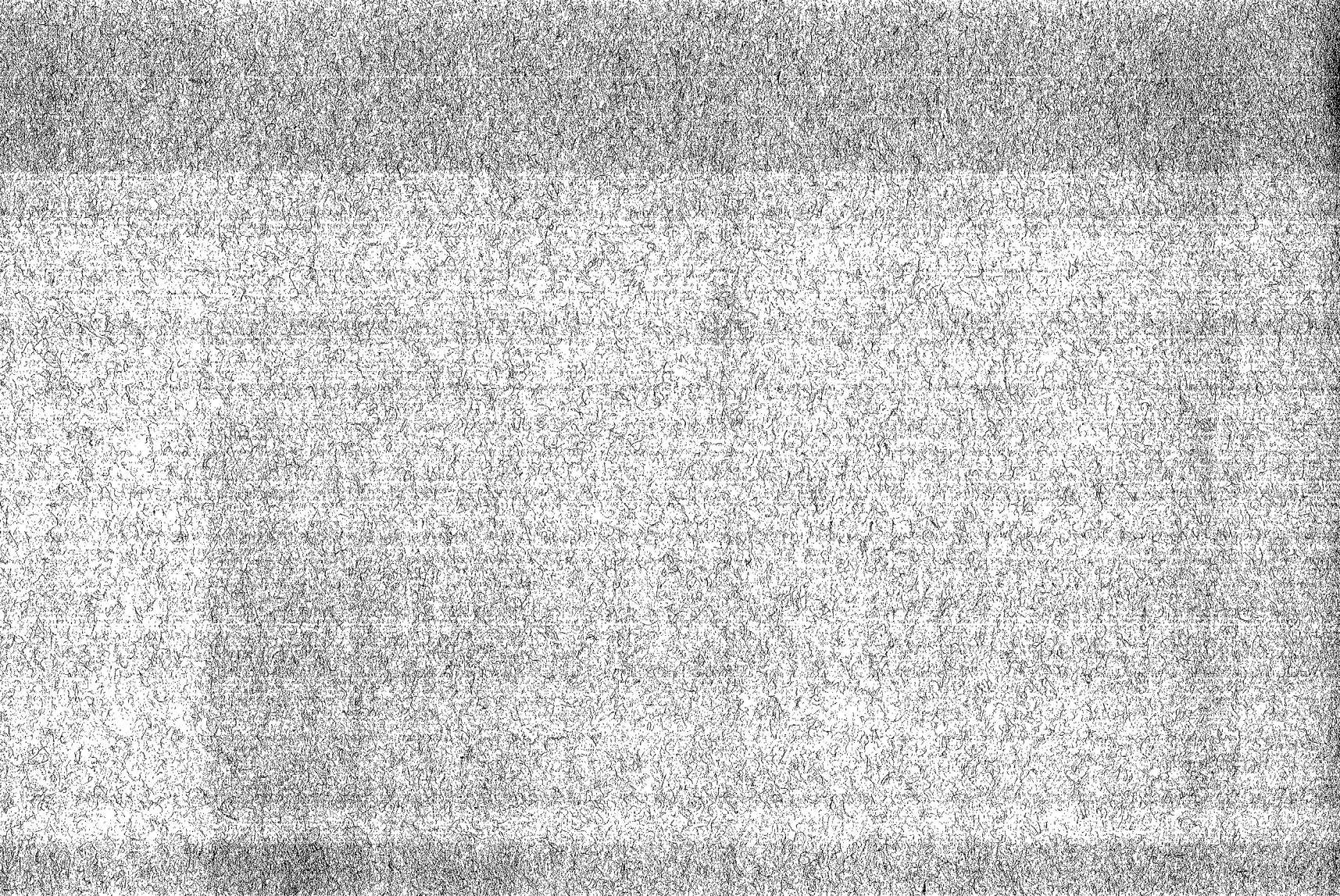
RECUEIL DES TRAVAUX CHIMIQUES DES PAYS-BAS

edited by the Koninklijke Nederlandse Chemische Vereniging

[The Hague, Holland]

T 74 — No. 1 — Jan. 1955

D. B. CENTEN's Uitgevers-Maatschappij, Amsterdam



547.541.1 : 547.655.1 : 547.857.4

THE INFLUENCE OF CAFFEINE ON THE COUPLING  
REACTION BETWEEN  $\beta$ -NAPHTHOL AND  
 $p$ -DIAZOBENZENESULPHONIC ACID.

BY

J. TH. G. OVERBEEK, C. L. J. VINK \*), and H. DEENSTRA

(van 't Hoff-Laboratory and Clinic for Internal Medicine  
University of Utrecht, Netherlands).

The rate of the coupling reaction between  $\beta$ -naphthol and  $p$ -diazobenzenesulphonic acid is lowered by addition of caffeine. This effect can be explained by complex formation between caffeine and the two reactants. The complex between caffeine and  $\beta$ -naphthol is of the 1—1 type; that between caffeine and  $p$ -diazobenzenesulphonic acid probably also. Complexity constants have been determined. In the presence of caffeine the reaction follows two (or three) separate paths, one involving an activated complex consisting of one  $\beta$ -naphtholate ion and one diazonium ion, and one (or two) involving an activated complex consisting of one molecule of each of the reactants and one molecule of caffeine. Rate constants have been determined at 20° C, using a colorimetric technique.

### Introduction.

Conant and Peterson<sup>1)</sup> and Wistar and Bartlett<sup>2)</sup> have shown that the coupling reaction between diazonium salts and aromatic amines, phenols or naphthols is of the first order in the two components and, in intermediate pH regions, proportional to the concentration of OH<sup>-</sup> ions.

Recently the understanding of the coupling mechanism has been improved by the work of Zollinger and Büchler<sup>3)</sup> and by that of Pütter<sup>4)</sup>. A recent review on these reactions has been given by Zollinger<sup>5)</sup>. The most probable mechanism is a bimolecular reaction between the diazonium ion and a phenolate or naphtholate ion (or an

\*) Present address: Pediatric Department, University of Leyden.

<sup>1)</sup> J. B. Conant and W. D. Peterson, J. Am. Chem. Soc. 52, 1220 (1930).

<sup>2)</sup> R. Wistar and P. D. Bartlett, J. Am. Chem. Soc. 63, 413 (1941).

<sup>3)</sup> H. Zollinger and W. Büchler, Helv. Chim. Acta 34, 591 (1951).

<sup>4)</sup> R. Pütter, Angew. Chem. 63, 188 (1951).

<sup>5)</sup> H. Zollinger, Chem. Revs. 51, 374 (1952).

amine molecule). We could confirm this mechanism for the reaction between  $\beta$ -naphthol and p-diazobenzenesulphonic acid between pH 3 and 6.

At very high pH's (12—14) the rate of the reaction, instead of being proportional to the  $\text{OH}^-$  ion concentration, is inversely proportional to its square or its cube<sup>6)</sup>. This is explained by considering that at these high pH's most of the diazonium salt is converted into the diazotate or the nitrosoamine, but that only the small equilibrium concentration of the diazonium ion is active in the reaction mechanism.

We investigated this coupling reaction as a simple analogue of the diazo reaction of bilirubin<sup>7)</sup>. This last reaction apparently is quite sensitive to changes in the reaction medium. Consequently our attention was drawn to analogous influences in the case of the simpler reaction.

A number of monosaccharides, disaccharides, glycerol, urea, guanidin, sodium benzoate, potassium phthalate, and T-pol (a sulphuric ester type detergent) did not change the reaction rate significantly at pH 4.90 and at a concentration of 0.06 molar. Addition of caffeine, however, decreased the rate considerably. As this substance also has a pronounced influence on the coupling reaction with bilirubin, we investigated the effect more closely.

#### Reaction rates in media containing caffeine.

All the reactions were carried out at 20° C in a 0.15 molar sodium acetate-acetic acid buffer of pH = 5.09.

The coupling product of the reaction between  $\beta$ -naphthol and p-diazobenzenesulphonic acid is  $\beta$ -naphthol orange. The kinetics of the reaction can therefore conveniently be determined colorimetrically. For most of our measurements we used the Zeiss Pulfrich Photometer with filter S50. The molecular extinction coefficient of  $\beta$ -naphthol orange with this filter is 22,400 below pH = 10 and drops to 12,000 between pH 10 and 14.

To 7 ml of the buffer-caffeine- $\beta$ -naphthol solution 1 ml of the diazo reagent was added. This reagent was prepared as follows: Solutions with equimolar quantities of  $\text{NaNO}_2$  (tested with a  $\text{KMnO}_4$  solution) and sulphanilic acid were mixed. The end concentration of HCl in this reagent was: 0.05 molar.

The  $\beta$ -naphthol and the caffeine were pure products of melting points 121° C and 235° C respectively (literature<sup>8)</sup> 122° C and 235—237° C). The purity of the sulphanilic acid was tested colorimetrically after diazotization with a slight excess of  $\text{HNO}_3$ , followed by coupling with  $\beta$ -naphthol.

<sup>6)</sup> C. L. J. Vink, thesis Utrecht 1954, p. 20.

<sup>7)</sup> A. A. Hijmans van den Bergh and I. Snapper, *Deut. Arch. klin. Med.* **110**, 540 (1913); A. A. Hijmans van den Bergh and P. Muller, *Biochem. Z.* **77**, 90 (1916); cf. C. L. J. Vink, l.c.

<sup>8)</sup> C. D. Hodgman, *Handbook of Chemistry and Physics*, 30th ed. 1947. Chemical Rubber Publishing Co., Cleveland Ohio, pp. 715 and 933.

In the reaction mixture the concentrations of  $\beta$ -naphthol and of *p*-diazobenzenesulphonic acid were chosen equal, both 0.0005 molar. As the pH is sufficiently buffered, a second-order rate constant,  $k$ , could be calculated from the following equation:

$$k = \frac{1}{t} \frac{c_d}{c_0(c_0 - c_d)} = \frac{1}{t} \frac{E}{c_0(E_\infty - E)} \text{ mole}^{-1} \text{ l min}^{-1} \quad (1)$$

where  $t$  is the time in minutes,  $c_d$  the molar concentration of the dye, and  $c_0$  the initial concentration of the reactants.  $E$  is the extinction measured at time  $t$ ,  $E_\infty$  the extinction at completion of the reaction.

This equation kept its validity in the presence of caffeine (table I); the order of the reaction is not changed, although the rate is modified considerably, as is shown in table II.

Table I.

Second-order rate constants of the coupling between  $\beta$ -naphthol and *p*-diazobenzenesulphonic acid (both 0.0005 molar) in the presence of 0.0273 molar caffeine at pH = 5.09 and at 20° C.

Reaction time in minutes	Rate constant $k$ in $\text{mole}^{-1} \text{ l min}^{-1}$
1	142
2	145
5	142
10	148

Table II.

Second-order rate constants of the coupling between  $\beta$ -naphthol and *p*-diazobenzenesulphonic acid (both 0.0005 molar) in the presence and in the absence of caffeine.

pH = 5.09		T = 20° C	
Concentration caffeine in moles/l.		Rate constant $k$ in $\text{mole}^{-1} \text{ l min}^{-1}$	
0		385	
0.00171		342	
0.00342		315	
0.00683		269	
0.01365		207	
0.0273		144	
0.0547		101	
0.1094		72	

In using extinction data for determining the rate constant, it was necessary to take into account the influence of caffeine on the extinction of  $\beta$ -naphthol orange. The value of  $E_\infty$  in eq. (1) depends on the amount of caffeine present.

The influence of caffeine on the reaction rate is most naturally explained by assuming complex formation between caffeine and  $\beta$ -naphthol and/or p-diazobenzenesulphonic acid.

#### Complex of caffeine and $\beta$ -naphthol.

The complex formation between caffeine and  $\beta$ -naphthol was studied by determining the increase of the solubility of  $\beta$ -naphthol in the presence of caffeine. Suitable mixtures were agitated during 5 to 8 hours in a water bath at 20° C till equilibrium was attained. The amount of solid  $\beta$ -naphthol was kept below 2 mg per ml of solution, in order to avoid complications through complex formation in the solid state. The concentration of  $\beta$ -naphthol was determined colorimetrically after complete reaction with p-diazobenzenesulphonic acid. The amount of  $\beta$ -naphthol present in the complex was calculated by subtracting the solubility in water from the solubility in the presence of caffeine.

The solubility of caffeine in water and in a solution saturated with  $\beta$ -naphthol was found by determining the total quantity of caffeine added to the solution, till a very small excess of this compound (needle-shaped crystals) did not disappear between the  $\beta$ -naphthol plates after some hours. Table III gives these solubilities.

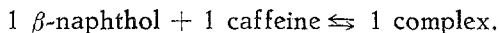
Table III.  
Solubilities of  $\beta$ -naphthol in the presence of caffeine at 20° C.

Concentration caffeine in $10^{-2}$ mole/l.	Saturation concentration $\beta$ -naphthol in $10^{-2}$ mole/l.	Complex $\beta$ -naphthol in $10^{-2}$ mole/l.	Complex caffeine in $10^{-2}$ mole/l.	Equilibrium constant $K_N$ in l/mole.
14.4 (saturated)	4.05 (extrapolated)	3.63 (extrapolated)	3.6	80.4
10.94	3.19	2.77		81.1
5.47	1.82	1.40		82.1
2.73	1.09	0.67		77.8
1.365	0.76	0.34		79.8
0	0.418	0		—
10.8 (saturated)	0 (no $\beta$ -naphthol present)			—
				average $K_N = 80.2$ l/mole s.d. = 1.6 l/mole

The fact that the concentrations of complex  $\beta$ -naphthol and of complex caffeine are equal in the solution that is saturated with respect to both solids, proves that the complex contains equal amounts of  $\beta$ -naphthol and caffeine.

The proportionality of complex  $\beta$ -naphthol and total caffeine shows that a constant fraction of the caffeine is present in the complex. This is only in agreement with a 1-1 complex. An  $n$ - $n$  complex with  $n$  larger than 1 would result in a larger fraction of the caffeine, complexed at larger total caffeine concentration.

Consequently the equation for the complex formation is



the equilibrium constant being

$$K_N = \frac{[\text{complex}]}{[\beta\text{-naphthol}] [\text{caffeine}]} \dots \dots \dots (2)$$

The equilibrium constants calculated with neglect of activity corrections are given in the last column of table III.

The average value of  $K_N$  is

$$\overline{K_N} = 80.2 \text{ l/mole.}$$

**Complex of caffeine and *p*-diazobenzenesulphonic acid.**

The composition and the equilibrium constant for the complex between caffeine and *p*-diazobenzenesulphonic acid were determined by the same method.

The *p*-diazobenzenesulphonic acid was prepared according to Schmitt<sup>9)</sup>.

The solubility data are given in table IV.

Table IV.

Solubilities of *p*-diazobenzenesulphonic acid in the presence of caffeine at 20° C.

Concentration caffeine in 10 <sup>-2</sup> mole/l.	Saturation concentration of <i>p</i> -diazobenzenesulphonic acid in 10 <sup>-2</sup> mole/l.	Complex <i>p</i> -diazobenzenesulphonic acid in 10 <sup>-2</sup> mole/l	Complex caffeine in 10 <sup>-2</sup> mole/l.	Equilibrium constant $K_D$ in l/mole.
11.6 (saturated)	—	—	0.8	1.19
10.80	7.01	0.79		1.27
9.31	6.82	0.60		1.11
9.00	6.90	0.68		1.31
0	6.22	—		—

average  $K_D = 1.22 \text{ l/mole.}$   
s.d. = 0.09 l/mole.

<sup>9)</sup> R. Schmitt, Ann. 190, 76 Ann. 2 (1877).

The complexity constant is evidently much smaller than that with  $\beta$ -naphthol, and its determination therefore involves considerable uncertainty. The values in columns 3 and 4 of table IV indicate that the complex contains equimolecular amounts of the two components. It cannot, however, be concluded with certainty that the complex is a 1-1 one, although this is the most probable interpretation of the data.

The average value of the equilibrium constant

$$K_D = \frac{[\text{complex}]}{[\text{p-diazobenzenesulphonic acid}][\text{caffeine}]} \quad (3)$$

is calculated as

$$\overline{K_D} = 1.22 \text{ l/mole.}$$

#### Interpretation of the reaction rates.

The coupling reaction may be considered to follow a number of different paths. We will consider the reactions

- a. between free  $\beta$ -naphthol and free p-diazobenzenesulph. acid
- b. between complex  $\beta$ -naphthol and free p-diazobenzenesulph. acid
- c. between free  $\beta$ -naphthol and complex p-diazobenzenesulph. acid
- d. between complex  $\beta$ -naphthol and complex p-diazobenzenesulph. acid

These four reactions are all compatible with the second-order course of the reaction. The total rate constant  $k$  mentioned in table II should then be related in the following way to individual rates

$$k(n)(d) = k_1 [N][D] + k_2 [NC][D] + k_3 [N][DC] + k_4 [NC][DC] \quad (4)$$

where (n) and (d) represent total concentrations of naphthol and of the diazonium salt, [N] and [D] the free concentrations of these compounds, [NC] and [DC] the concentrations of their caffeine complexes.

Using the equilibrium conditions (1) and (2) to eliminate [N], [D], [NC], and [DC] and taking into account that in all the experiments mentioned in table II only a very small fraction of the caffeine is involved in the complex formation, we find

$$\frac{(k-k_1) + k(K_N + K_D)[C] + k(K_N K_D)[C]^2}{[C]} = (k_2 K_N + k_3 K_D) + k_4 K_N K_D [C] \quad (5)$$

In fig. 1 the lefthand member of eq. (5), which can be calculated from the measurements, is plotted against [C], the concentration of



caffeine. The points at high caffeine concentration are by far the most reliable ones. The vertical lines indicate the possible error introduced by a 2% error in the reaction constants.

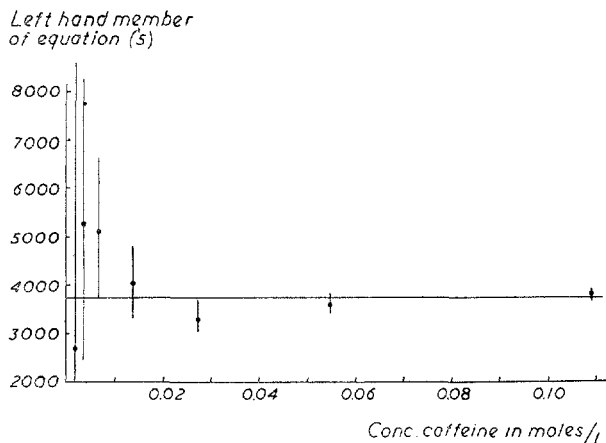


Fig. 1. Graph for determining the reaction rates of the complexes (see text).

Evidently the best straight line through the points is a horizontal line, indicating that the reaction rate between the two complexes is very small. A maximum value of  $k_4$  compatible with our results would be  $k_4 \leq 100 \text{ mole}^{-1} \text{ l min}^{-1}$ . The value of  $k_2K_N + k_3K_C$  is found to be  $3700 \text{ mole}^{-2} \text{ l}^2 \text{ min}^{-1}$ .

It is not possible to determine  $k_2$  and  $k_3$  separately from the available data. This is necessarily so, because the two reactions both pass along the same activated complex, built up from one phenolate ion, one diazonium ion, and one caffeine molecule.

One may of course assume that the reaction follows only one of the two possible paths. This amounts to arbitrarily choosing either  $k_2$  or  $k_3$  equal to zero. In that case the other reaction constant can be calculated. In this way we find that, if the reaction is exclusively one between the complex  $\beta$ -naphthol and the free diazonium ion, the rate constant  $k_2$  is equal to

$$k_2 = 46.1 \text{ mole}^{-1} \text{ l min}^{-1},$$

and if the reaction occurs exclusively between the free naphtholate ion and the diazonium complex,

$$k_3 = 3030 \text{ mole}^{-1} \text{ l min}^{-1}.$$

Comparing these values with the rate constant for the free naphtholate with the free diazonium ion  $k_1 = 385 \text{ mole}^{-1} \text{ l min}^{-1}$ , it appears

that complexing the naphthol with caffeine decreases the reaction rate quite considerably, while the complexed diazonium ion *may*, but must not, react faster than the free ion. The available data do not allow of any further analysis of this phenomenon of interaction.

**Acknowledgement.**

The support of the Netherlands organization for pure research (Z.W.O.) is gratefully acknowledged.

*(Received August 21st 1954).*

