(Asahina) which contains squamatic and baecomycesic acids and which shows a quite distinct bright white fluorescence. By extracting the pure lichen (10 g) with ether in a Soxhlet-apparatus, crude thamnolic acid (1 g) was obtained. It was chromatographically indistinguishable from ocellatic acid and was obtained from dioxan as prisms containing one mole of dioxan, lost after drying *in vacuo* at 100°. Found: Loss of weight: 17.0 %. $C_{19}H_{16}O_{11}$, $C_8H_8O_2$ requires 17.3 % dioxan. Found: Equiv.wt. 205. $C_{19}H_{16}O_{11}$ requires equiv.wt. 210.1.

The author is indebted to Docent R. Santeson, Botaniska Museet, Uppsala, for collecting the *Thamnolia* mixture and to Fil.lic. K. E. Almin, Svenska Träforskningsinstitutet, Stockholm, for performing the infra-red spectra.

1. Hesse, O. J. *prakt. Chem.* **63** (1901) 551.
2. Asahina, Y. and Shibata, S. *Chemistry of lichen substances*, Tokyo 1954.
3. Wachtmeister, C. A. *Acta Chem. Scand.* **6** (1952) 818.
4. Koller, G. and Hamburg, H. *Monatsh.* **65** (1935) 375.
5. Asahina, Y. Aoki, M. and Fuzikawa, F. *Ber.* **74** (1941) 828.

Received September 9, 1955.

The Standard Oxidation Potential of the System, 1,4-Naphthoquinone/H⁺ — 1,4-Naphthohydroquinone and the Solubility of 1,4-Naphthohydroquinone, 1,4-Naphthoquinone and 1,4-Naphthoquinhydrone

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Previously LaMer and Baker have determined by electrometric titration the standard oxidation potential of the system

1,4-naphthohydroquinone = 1,4-naphthoquinone + 2H⁺ + 2e at 25° C. They found the value 0.4698 ± 0.0002 volt¹. Many other values are reported in the literature, but they have all been determined in non-aqueous solutions, and are therefore incomparable to the value here reported.

The solubility of 1,4-naphthohydroquinone, 1,4-naphthoquinone and 1,4-naphthoquinhydrone has not been determined before. The solubilities of 1,4-naphthoquinone and 1,4-naphthohydroquinone presented in this paper have been determined directly, that of 1,4-naphthoquinhydrone has been calculated as shown below.

The standard oxidation potential presented in this paper for the system in question at 20° C in an aqueous solution of 0.01 M HCl + 0.09 M KCl was determined in two ways (see Table 1) combined with the determination of the solubility of 1,4-naphthoquinone and of 1,4-naphthohydroquinone.

Denoting the electromotive forces of the three cells by E_A , E_B and E_C , respectively we have the following equations: (the liquid-liquid junction potential being taken as zero)

$\pi_0' - E_A = \pi_0$, $\pi_0' - E_B = \pi_B$ and $\pi_0' - E_C = \pi_C$, where π_0' and π_0 are the standard oxidation potentials of the systems benzoquinone—benzohydroquinone and 1,4-naphthoquinone — 1,4-naphthohydroquinone, respectively; π_B and π_C are the oxidation potentials at pH = 0 in the left half-cells of (B) and (C). π_0 and the solubility product of 1,4-naphthoquinhydrone L may be calculated from π_B , π_C and the solubilities of 1,4-naphthoquinone (c_{OQu}) and 1,4-naphthohydroquinone (c_{OHy}):

$$\begin{aligned}\pi_B &= \pi_0 + 0.02905 \log c_{OQu}/c_{Hy} \text{ and} \\ \pi_C &= \pi_0 + 0.02905 \log c_{Qu}/c_{OHy} \text{ and} \\ c_{Qu}c_{OHy} &= c_{OQu}c_{Hy} = L\end{aligned}$$

c_{Hy} and c_{Qu} are the concentrations of 1,4-naphthohydroquinone and 1,4-naphthoquinone in the left half-cells of (B) and (C), respectively. The following results were obtained: (π_0' is taken as 0.7028 volt²) $c_{OQu} = (1.099 \pm 0.005) \cdot 10^{-3}$ mole/liter, $c_{OHy} = (5.25 \pm 0.03) \cdot 10^{-3}$ mole/liter. $E_A = 0.2211 \pm 0.0005$ volt, $E_B = 0.2092 \pm 0.0001$ volt, $E_C = 0.2735 \pm 0.0001$ volt. The value of π_0 calculated by means of π_0' and E_A is 0.4817 ± 0.0005 volt. The value of π_0 calculated by means of π_0' , E_B , E_C , c_{OQu} , and c_{OHy} is 0.4812 ± 0.0002 volt. The calculated value for L and the solubility of 1,4-naphthoquinhydrone are

Table 1. The standard oxidation potential was determined in two ways:

1) by measuring the electromotive force of the cell (A)

Pt	1,4-Naphthoquinhydrone (cryst.) 0.01 M HCl + 0.09 M KCl	0.01 M HCl 0.09 M KCl	Benzoquinhydrone (cryst.) 0.01 M HCl + 0.09 M KCl	Pt	(A)
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2) by measuring the electromotive force of the cells (B) and (C)

Pt	1,4-Naphthoquinone (cryst.) 1,4-Naphthoquinhydrone (cryst.) 0.01 M HCl + 0.09 M KCl	0.01 M HCl 0.09 M KCl	Benzoquinhydrone (cryst.) 0.01 M HCl + 0.09 M KCl	Pt	(B)
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and

Pt	1,4-Naphthoquinhydrone (cryst.) 1,4-Naphthohydroquinone (cryst.) 0.01 M HCl + 0.09 M KCl	0.01 M HCl 0.09 M KCl	Benzoquinhydrone (cryst.) 0.01 M HCl + 0.09 M KCl	Pt	(C)
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$(4.51 \pm 0.07) \cdot 10^{-7}$ (mole/liter)² and $(6.72 \pm 0.05) \cdot 10^{-4}$ mole/liter, respectively.

Experimental. Chemicals (all melting points uncorrected):

1,4-Naphthoquinone was purified by distillation with steam and recrystallisation from ethanol; m. p. 125°—126° C.

1,4-Naphthohydroquinone was prepared by reduction of 1,4-naphthoquinone with stannous chloride and recrystallisation from water. The product was preserved in a sealed glass tube filled with nitrogen; m. p. 191°—192° C.

1,4-Naphthoquinhydrone was prepared by mixing concentrated solutions of 1,4-naphthoquinone and 1,4-naphthohydroquinone in ethanol. The product was recrystallised from ethanol, and preserved in a sealed glass tube filled with nitrogen.

Benzoquinhydrone was recrystallised from ethanol.

The solubility measurements.

The solubility of 1,4-naphthoquinone. The aqueous solution of 0.01 M HCl + 0.09 M KCl was transferred into two 250 ml flasks each of which was closed with a rubber stopper mounted with a Jena 33 G 4 gas filter dipping in the solution, and a glass tube with a stopcock. In order to free the solution from oxygen, carbon dioxide was led through for about 30 min. The carbon dioxide was washed in a titanium trichloride solution containing a citrate buffer. After the removal of oxygen 0.5 g of 1,4-naphthoquinone was suspended in each flask.

In order to reach equilibrium both from the side of supersaturation and from the side of subsaturation one of the flasks was kept at

40° C for one hour before being placed in the thermostat, where they were rotated at $20.00 \pm 0.02^\circ$ C. With aid of carbon dioxide under pressure samples were obtained by forcing the solution through the filter into a 15 ml automatic Knudsen-pipette. As burette was used a Krogh-syringe mounted with a micrometer screw. The content of quinone was titrated with titanium trichloride and indigodisulfonate as indicator.

Samples were analysed after the lapse of 4 hours, 25 hours, 48 hours and 72 hours. The process, supersaturation — saturation, proceeds very slowly. It took between 48 and 72 hours before equilibrium was reached. On the other hand the equilibrium of the process, subsaturation — saturation, is reached in the course of 4 hours.

The solubility of 1,4-naphthohydroquinone. The same procedure as that described above was used, except that equilibrium was established only from the side of subsaturation. The content of hydroquinone was titrated with ammonium ferriessulfate and potassium rhodanide as indicator.

The electrometric measurements. The apparatus are earlier described³. A Wolff compensator with a mirror galvanometer was used. The position of the mirror was read by telescope. As standard cells were used two Weston cells compared to each other. The sensibility was 0.1 mV.

Care was taken to avoid oxygen in the cells. The electrodes were washed with the aqueous solution of 0.01 M HCl + 0.09 M KCl. This was freed from oxygen by bubbling-through nitrogen which previously had been passed

over pieces of copper wire heated to some 500° C.

The electromotive forces of the cells and the time passed since the last washing of the electrodes were measured. Cell (B) and (C) became constant in 20 hours since the last washing of the left electrodes had been effected.

The work has been completed at Laboratoriet for Fotokemi, Fotografi og Reproduktionsteknik, Technical University, Copenhagen. The authors thanks are due to dr. E. Güntelberg for information and help in the work.

1. LaMer, V. K. and Baker, L. E. *J. Am. Chem. Soc.* **44** (1922) 1960.
2. Biilmann, E. *Trans. Faraday Soc.* **19** (1923) 677.
3. Güntelberg, E. and Schiødt, E. *Z. physik. Chem.* **135** (1928) 393.

Received September 10, 1955.

Paper Chromatography of Nucleoside Polyphosphates

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Paper chromatography according to the method of Hanes and Isherwood¹, namely descending chromatography with *n*-propanol-ammonia (*d* 0.880)-water (60:30:10) separates the 5'-mono-, di- and triphosphates of any one of the ribonucleosides adenosine, guanosine, inosine, cytidine and uridine from each other. On the other hand, difficulties are generally encountered on attempts to separate by this method mixtures which contain phosphates of the different nucleosides. Some time ago, the need arose in this laboratory for the resolution of mixtures containing different adenosine and inosine 5'-phosphates and a method for the separation of such mixtures by two-dimensional paper chromatography was devised². In the first direction, the chromatogram was developed with *n*-propanol-ammonia-water, separating the individual members in each series and in the second direction with a solvent system consisting of saturated ammonium sulphate solution-water-*iso*-propanol (79:19:2), which had been introduced³ for the separation of the pro-

Fig. 1. Two-dimensional chromatogram (Whatman No. 1 paper) of a mixture of AMP, ADP, ATP, GMP, GDP, GTP, IMP, IDP, ITP, UMP, UDP and UTP, approximately 50 µg of each compound. (A) first solvent: *n*-propanol-ammonia-water (60:30:10), 60 hours. (B) second solvent: saturated ammonium sulphate solution-water-*iso*-propanol (79:19:2), 10 hours. (1) AMP; (2) ADP; (3) ATP; (4) GMP; (5) GDP; (6) GTP; (7) IMP; (8) IDP; (9) ITP; (10) UMP; (11) UDP; (12) UTP.

ducts of hydrolysis of nucleic acids. It was found that this solvent separates the two nucleoside series of phosphates from each other.

In the course of recent work on the isolation of the 5'-triphosphates of guanosine^{4,5} and uridine^{4,6} from muscle, the ammonium sulphate-water-*iso*propanol solvent system proved to be useful for the separation of the 5'-triphosphates of different nucleosides including those of guanosine, uridine, adenosine, inosine and xanthosine. We have therefore applied the previously described method of two-dimensional paper chromatography to the resolution of mixtures containing the 5'-mono-, di- and triphosphates of adenosine (AMP, ADP and ATP), guanosine (GMP, GDP and GTP), inosine (IMP, IDP and ITP) and uridine (UMP, UDP and UTP) and obtained adequate resolution of all the twelve components (Fig. 1). On the other hand,