evaporated *in vacuo*. The residue was dissolved in methanol and a little water. Ether was added, and the amorphous precipitates, which formed regularly, were discarded. Crystalline deposits were obtained in a few days. These were purified by recrystallization from a water-methanol mixture.

Some analytical data of the crystalline material are given in Table 2.

Table 2.

Nitrogen	4.38 %
Acetyl	14.96 %
Methoxyl	0
Decomposition-point	185—187° (uncorr.)
Optical rotation	$[\alpha]_D^{23} = -32^\circ \pm 2^\circ$

The X-ray diffraction pattern is seen in Fig. 1. It is identical with that of sialic acid isolated from sheep's submaxillary mucin.

The same sialic acid was isolated from the pseudomyxomatous contents of two other cysts, and from the peritoneal gel of a pseudomyxoma peritonei also present in one of the cases. (The contents of a mucocele appendicis in the last case had the same composition as the other gels. Isolation of sialic acid was not attempted because of lack of material.)

A detailed report of this work will be published elsewhere.

1. Morgan, W. T. J. and van Heyningen, R. *Brit. J. Exptl. Pathol.* **25** (1944) 5.
2. Bray, H. G., Henry, H. and Stacey, M. *Biochem. J. (London)* **40** (1946) 124.
3. Bendich, A., Kabat, E. A. and Bezer, A. E. *J. Am. Chem. Soc.* **69** (1947) 2163.
4. Aminoff, D. and Morgan, W. T. J. *Nature* **162** (1948) 579.
5. Hiyama, N. *Tohoku J. Exptl. Med.* **51** (1949) 327.
6. Jensen, C. E. *Acta Pharmacol. Toxicol.* **10** (1954) 83.
7. Blix, G., Lindberg, E., Odin, L. and Werner, I. *Nature* **175** (1955) 340.
8. Gardell, S. *Acta Chem. Scand.* **7** (1953) 207.
9. Vasseur, E. *Acta Chem. Scand.* **2** (1948) 693.
10. Werner, I. and Odin, L. *Acta Soc. Med. Upsaliensis* **57** (1952) 230.
11. Blix, G. *Hoppe-Seylers Z. physiol. Chem.* **240** (1936) 43.

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A New Method for Peroxide Determination

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In a previous paper¹ vanillin dissolved in 70 % sulphuric acid was shown to be a qualitative reagent on peroxides. In trying to apply this test for quantitative analysis we found that with regard to colour production, hydrogen peroxide differs from the tertiary butyl peroxides $(H_3C)_3C-OOH$ and $(H_3C)_3C-OO-C(CH_3)_3$. Hydrogen peroxide (Fig. 1) gives a green colour, only slightly dependent on the added amount of peroxide. Thus this colour reaction can not be used as a quantitative reagent. Moreover, the colour is not sufficiently different from that of aged solutions of protocathecuic aldehyde (demethylated vanillin) or piperonal (vanillin - 2 H) in 70 % sulphuric acid.

The tertiary butyl peroxides also colours vanillin-sulphuric acid green, but after a few hours the colours shift over violet into deep blue (Fig. 1). The blue reaction products (called "vanillin-blue" in this paper) precipitate on diluting the sulphuric acid solution with ice water, preferably with at least five volumes. The precipitate dissolves completely without impairment of the colour in concentrated acids, like sulphuric, phosphoric, acetic or formic. Hydrochloric acid should be avoided as evolution of chlorine was observed. Organic bases and alkali destroy vanillin-blue. The precipitate was washed with ice water until the pH of the water was 5, and dried *in vacuo* over $CaCl_2$. In standing at room temperature, vanillin-blue changes colour and solubility.

The formation of vanillin-blue increases with temperature. As the peroxides and vanillin-blue itself, however, decompose on heating, it will be an optimum for the reaction. According to preliminary experiments, this optimum is about 37° C. There are also many practical reasons to use this temperature, and all data given here are obtained by heating the samples at 37° C. The formation of vanillin-blue is slower at higher concentrations. For practical reasons the reaction was stopped after 20 hours. Consequently, the dosage curves will not be straight lines but curved downwards.

Fig. 1. Extinction curves for vanillin-sulphuric acid treated with:

a. Hydrogen peroxide (1×10^{-4} M) — — — — —. b. *t*-BuOOH (1.7×10^{-5} M), diluted with ethanol — — — — —. c. *t*-BuOOH, precipitated, and dissolved in formic acid — — — — —

The most accurate method of analysis for *t*-butyl peroxides, based on formation of vanillin-blue, will be to filter off the precipitate, wash and dissolve it in formic acid, and determine the extinction at 600 $m\mu$. By this treatment coloured impurities disturbing the measurements are removed. Already the pure reagent itself strongly colours the supernatant solution brilliant red (abs.-max. at 500 $m\mu$). The particle size of the vanillin-blue precipitate, however, is so that about 30 % goes through glass filter G 4. In using a finer filter good results were obtained, but the time for filtering will be too long for practical use. The old trick of clotting colloidal precipitates of this kind by heating fails here, since vanillin-blue is heat-sensitive.

Compounds showing extinction at 600 $m\mu$ are rather uncommon, and hence direct determination of the reaction product will be convenient. Generally the colouration is so strong that the solutions have to be diluted before extinctionometry. A dilution with 70 % sulphuric acid shifts the absorption maximum over to 560 $m\mu$, and the dosage curve (Fig. 2) is strongly bent. In using ethanol for dilution, the absorption maximum (Fig. 1) remains at 600 $m\mu$, and the dosage curve is straighter. With both kinds of dilution the colour remains constant for a couple of hours at room temperature, or for the sulphuric acid dilutions for a day in the ice-chest.

Method. To 1 ml, or less, of an aqueous solution containing *t*-butyl hydroperoxides or di-*t*-butyl peroxides, add 10 ml of a solution of 15.2 mg vanillin (10^{-3} M) in 70 % (by volume) sulphuric acid. After 20 h in a water bath at 37° C, dilute with 20 ml ethanol, and measure the extinction in a Klett-Summerson photometer using filter 60. At this dilution the method covers the concentration range 10^{-5} to 10^{-6} moles (1–100 μ g).

Discussion

The dosage curves approach straight lines when smaller amounts of peroxides than 10^{-6} moles are added to the vanillin-sulphuric acid. For analytical purposes it will be better to use this method at higher concentrations only for orientation. The great deviations from the dosage curves may be ascribed to errors at the dilution, which at 10^{-6} moles was 1:100. Another error may be due to the dilution of the sulphuric acid in mixing it with the sample. The formation of vanillin-blue is restricted to concentrations between 60 % and 80 % with an optimum at 70 %. If larger amounts than 1 ml are to be added to 10 ml vanillin-sulphuric acid, the acid must be more concentrated, so as to obtain 70 % after mixing.

In the optimal region (10^{-8} to 10^{-6} moles added peroxide), the extinction at 600 $m\mu$ is $(31.5 \pm 0.7) \times 10^3$ units/mole, for dilu-

Fig. 2. Dosage curves for vanillin-blue:

- a. Diluted with 70 % sulphuric acid $\circ$ — — — $\circ$
 b. Diluted with 96 % ethanol + — — — +

tion with ethanol, and $(28.4 \pm 0.9) \times 10^3$ units/mole, for sulphuric acid dilution. The advantage in diluting with ethanol is the maximal absorption at 600 $m\mu$. The interference with other coloured substances formed, *i. e.*, the red substance in the supernatant liquid will be less in ethanol. On the other hand the alcoholic solutions are less stable. A point to be noted in the practical application of the method is that both an aged vanillin-sulphuric acid solution and a freshly prepared one will give the same dosage curve.

For the use of vanillin-blue as a peroxide reagent, it is unnecessary to know its formula or the reaction mechanism. The amount of vanillin exceeds greatly that of the peroxides (ranging from 1:10 to 1:10⁴). It is doubtful whether the vanillin-blue formed and precipitated has a constant composition. However, an analysis was made, giving C 68.2, H 5.46, and O 24.3 (1 % ash).

Campbell and Coppinger² added *t*-butyl hydroperoxide to 2,6-di-*t*-butyl-*p*-cresol, and obtained a substance described as 1-methyl-1-*t*-butylperoxy-3,5-di-*t*-butylcyclohexadienone-4. With this type of peroxide (which have an absorption maximum at 234 $m\mu$) vanillin-blue seems to have little in common. We exclude a compound with the *t*-BuOO-group directly attached to the aromatic ring as such compounds will not be stable. In view of the small amounts of peroxide needed for the formation of colour and the unknown be-

haviour of less stable peroxides such as the methylhydroperoxide, no formula for vanillin-blue is advanced.

1. Arrhenius, S. *Svensk Kem. Tidskr.* **64** (1952) 260.
2. Campbell, T. W. and Coppinger, G. J. *Am. Chem. Soc.* **74** (1952) 1469.

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4s-Electrons in the First Transition Group Complexes

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When the crystal field theory is applied^{1,2} to the absorption spectra of hexaaquo ions and other simple complexes of the first transition group, small discrepancies ($\sim 5\%$) occur between the calculated and observed wavenumbers of the band maxima. Mainly based on magnetic evidence, Owen³ has interpreted these effects by $\sim 20\%$ intermixing of σ -electrons from the ligands with the γ_3 -electrons (the highest energy state of d-electrons in an octahedral complex while the other is γ_5). In the present note attention will be drawn to the electron configuration $3d^{n-1} 4s$, which in the free, divalent ion is situated⁴ $\sim 50\,000\text{ cm}^{-1}$ over the groundstate, due to $3d^n$.

Since these two electron configurations have the same parity (the sum of the l-values is even) they can directly intermix. The author⁵ even maintains that the crystal field induces an intermixing with $3d^{n-1}4p$ and electron transfer states, explaining the observed transitions which would else be forbidden by Laporte's rule. The former interaction will *inter alia* depress the energy of the levels with the same Γ_n as the lowest level of the excited $3d^{n-1} 4s$ configuration. Thus, in d^2 -systems, the levels $^4\Gamma_4$ will be depressed by the ground-state $\gamma_5^2\gamma_1$ of $3d^2 4s$. Now, Mr. C. E. Schäffer has kindly pointed out to me that the observed energy difference, $^4\Gamma_4 - ^4\Gamma_5$, between the two strong bands of chromium (III) complexes decreases by increasing crystal field strength, while it should⁶ be slightly increasing or constant, if no interaction appeared. Also V^{++} shows this effect if $^4\Gamma_5$ is placed at $12\,200\text{ cm}^{-1}$, contrary to Owen³.