We believe that the Co-TPP(THF)(TCNE) complex is salt-like in its solid state and is of the same structure as above, where the coordinated TCNE moiety is anion radical.

The interaction of chlorocarbonylbis(triphenylphosphine)iridium with TCNE was also investigated for comparison in benzene. The system gave rise to an isotropic ESR spectrum with 9 *hf* lines at room temperature (Fig. 3), which was similar to that observed in the reaction of Co-TPP with TCNE. The g value and the coupling constant ($g=2.0026$, $A_N=1.57$ G) were in good agreement with those of free TCNE anion radicals in solution. TCNE is thus believed to be π-bonded to the iridium complex in its solid state[4] likewise the TCNE adudct of bromocarbonylbis(triphenylphosphine)iridium.[8]

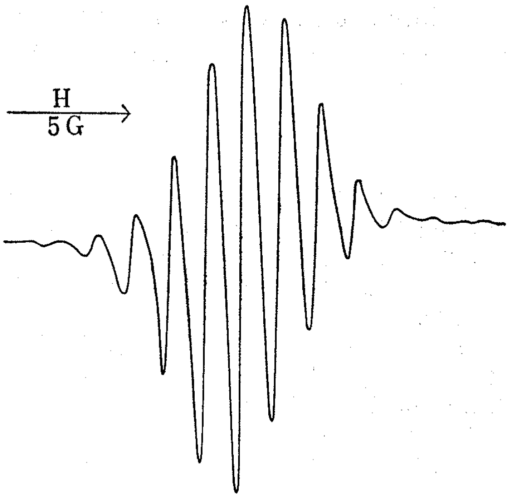

Fig. 3

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[8] J.A. McGinnety and J.A. Ibers, *Chem. Commun.*, **1968**, 235.

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UDC 547.455.02.05.08

A Colorimetric Determination of Sedoheptulose with Sulfuric Acid and Thioglycolic Acid[1]

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(Received June 22, 1972)

In our studies to determine the activity of transketolase (D-sedoheptulose-7-phosphate, D-glyceraldehyde-3-phosphate glycolaldehyde transferase E.C. 2.2.1.1),[3] it has been necessary to measure the accurate amount of sedoheptulose formed from the equilibrium mixture of ribose-5-phosphate, xylulose-5-phosphate and ribulose-5-phosphate. The determination of sedoheptulose with sulfuric acid and cysteine by the method of Dische[4] has generally been used for above purpose[5] and the slight modifications of this method have also been employed.[6,7]

We have investigated Dische's method for cutting the reaction time and increasing the specificity in determining sedoheptulose and found that the use of thioglycolic acid with sul-

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[1] This work was presented at the 24th Annual Meeting of the Vitamin Society of Japan, Koube, April 1972.

[2] Location: *2-2-1, Oshika, Shizuoka.*

[3] T. Ozawa, S. Saito, and I. Tomita, *Vitamins* (*Kyoto*), **44**, 303, 308 (1971).

[4] Z. Dische, *J. Biol. Chem.*, **204**, 983 (1953).

[5] T. Wood and W.M. Poon, *Arch. Biochem. Biophys.*, **141**, 440 (1970).

[6] G.R. Bartlett and G. Bucolo, *Biochim. Biophys. Acta.*, **156**, 240 (1968).

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furic acid at 50° completed the reaction in 30 minutes and the color development by this procedure from several pentoses such as arabinose, ribose, and xylose is almost of no significant influence upon sedoheptulose determination in transketolase reaction.

Experimental

Apparatus——Hitachi Perkin-Elmer spectrophotometer (UV-VIS 139) with Taiyo thermo unit (C 550) and Hitachi spectrophotometer (EPS-3-T) were used for the measurement of the optical density and recording the spectrum, respectively.

Reagents——Sedoheptulosan monohydrate and all SH containing reagents, used, were of guaranteed reagents.

Procedure——In Dische's method, 2.5 ml of 82% sulfuric acid and 0.05 ml of 3% cysteine hydrochloride solution (8.5 μmoles) were allowed to react in this order, with 0.5 ml of sample solution containing 10 μg (0.05 μmoles) of sedoheptulosan and the solution was kept in water bath of 20° for 24 hours. In modified method, 2.5 ml of 82% sulfuric acid and 0.05 ml of 0.68 molar solution of thioglycolic acid (34 μmoles) were used and heated at 50° for 30 minutes. Two methods employed above are outlined below for comparison.

Dische's procedure

test solution, 1 ml (0.5 ml)*
↓
addition of 82% H_2SO_4, 4.5 ml (2.5 ml)*
↓
kept at 0° for 3 min
20—25° for 3 min
100° for 3 min
↓
addition of 3% cysteine (0.17M),
0.1 ml (0.05 ml)*
↓
kept at room temperature for 24 hr

modified procedure

test solution, 0.5 ml
↓
addition of 82% H_2SO_4, 2.5 ml
↓
addition of 0.68M thioglycolic acid
0.05 ml (34 μmoles)
↓
kept in water bath at 50° for 30 min

* volumes in parentheses were conveniently used for comparison with modified procedure

Result

Fig. 1 shows the absorption spectra of the solutions produced from the reactions of sedoheptulosan with sulfuric acid and several SH containing reagents. The addition of 82% sulfuric acid and 3% cysteine solution to sedoheptulosan according to Dische's procedure with the reaction mixture being kept at 20° for 30 minutes did not complete the reaction. It must be kept for 24 hours for the completion. With 0.68M solution of cysteine (12% solution), and the incubation of the reaction mixture at 50°, however, completed the colour development in 30 minutes. Cysteine solution could be replaced by the equivalent molar solution of thioglycolic acid, glutathione, or ethylmercaptan. Wavelength of maximum absorption was varied with SH reagents used: 510 nm for both cysteine and thioglycolic acid, 515 nm for glutathione and 525 nm for ethylmercaptan. Time for the maximum color development was shortest with ethylmercaptan followed by thioglycolic acid, glutathione and cysteine.

Fig. 2 shows the absorption spectra of the solutions of various hexoses or pentoses treated with sulfuric acid and cysteine. All sugars used showed small but significant interfering absorptions at 510—540 nm region and $\Delta A_{510-540\ \mathrm{nm}}$ was 0.003 for ribose or arabinose and 0.018, 0.024, and 0.033 for glucose, fructose and xylose, respectively. It was 0.078 for galactose.

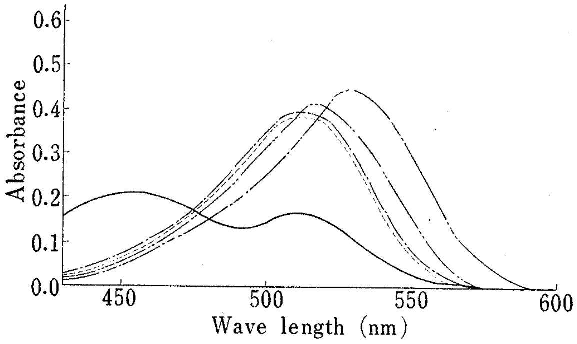

Fig. 1. Visible Absorption Spectra of the Colored Solution produced from 10 μg of Sedoheptulosan with H_2SO_4 and Several SH containing Reagents

———: cysteine 1.49 mg (8.5 μmoles), as cysteine·HCl·H_2O
- - - - - - -: cysteine 5.97 mg (34.0 μmoles), as cysteine·HCl·H_2O
——·——: thioglycolic acid 3.88 mg (34.0 μmoles), as sodium thioglycolic acid
——··——: glutathione 10.45 mg (34.0 μmoles)
—·—·—: ethylmercaptan 2.11 mg (34.0 μmoles)

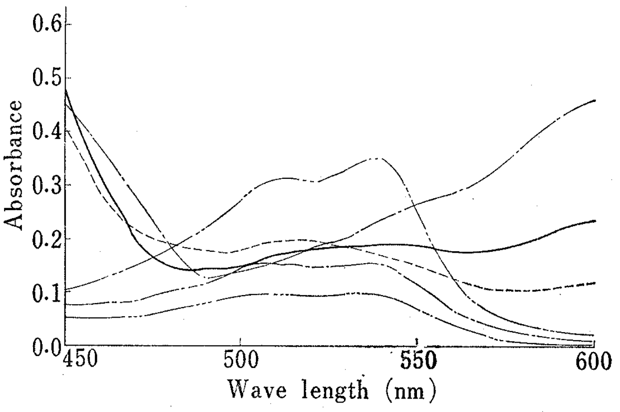

Fig. 2. Visible Absorption Spectra of the Colored Solution produced from Various Sugars with H_2SO_4 and Cysteine

———: glucose (100 μg)
- - - - - -: fructose (100 μg)
——·——: galactose (100 μg)
——··——: xylose (100 μg)
—·—·—: ribose (100 μg)
——···——: arabinose (100 μg)

The absorption spectra of the sugars treated with sulfuric acid and thioglycolic acid are shown in Fig. 3. It should be noted that the absorptions at 510 and 540 nm of the solutions produced from pentoses are much less than that shown in Fig. 2 and $\Delta A_{510-540\,\mathrm{nm}}$ are nearly 0. It is thus apparent from Fig. 3 that the use of thioglycolic acid is superior to cysteine for the determination of sedoheptulosan in the presence of pentoses. The reactions of sugars with sulfuric acid and other SH containing reagent such as glutathione or ethylmercaptan could also be used for the colorimetric determination of sedoheptulosan. The absorptions of sugars in the reaction with glutathione, however, are quite high, as shown in Fig. 4. The use of ethylmercaptan has also little advantage over that of thioglycolic acid or cysteine because of its unpleasant odour, though it produced no coloured substances with the various pentoses (Fig. 5). Fig. 6 shows the effect of time and the temperature for the incubation

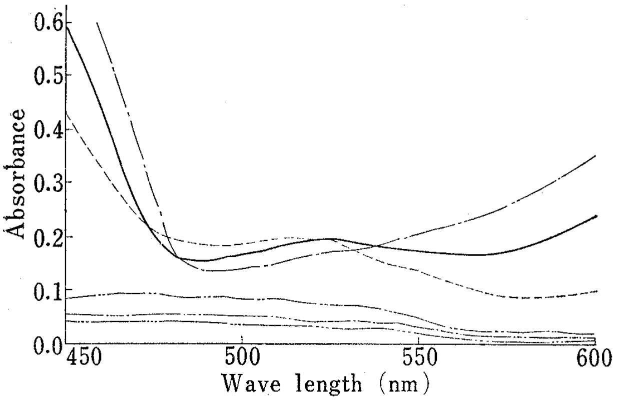

Fig. 3. Visible Absorption Spectra of the Colored Solution produced from Various Sugars with H_2SO_4 and Thioglycolic Acid

———: glucose (100 μg)
- - - - - - -: fructose (100 μg)
——·——: galactose (100 μg)
——··——: xylose (100 μg)
—·—·—: ribose (100 μg)
——···——: arabinose (100 μg)

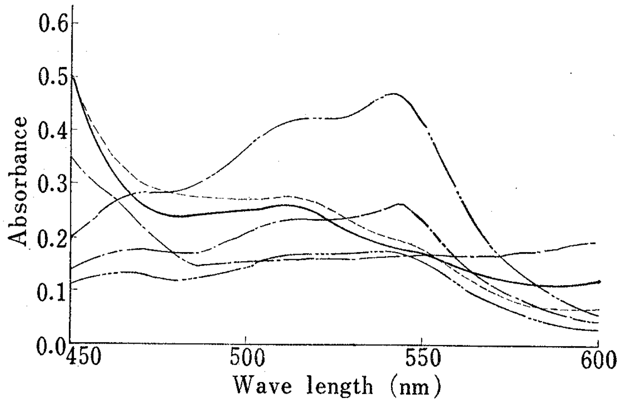

Fig. 4. Visible Absorption Spectra of the Colored Solution produced from Various Sugars with H_2SO_4 and Glutathione

———: glucose (100 μg)
- - - - - -: fructose (100 μg)
——·——: galactose (100 μg)
——··——: xylose (100 μg)
—·—·—: ribose (100 μg)
——···——: arabinose (100 μg)

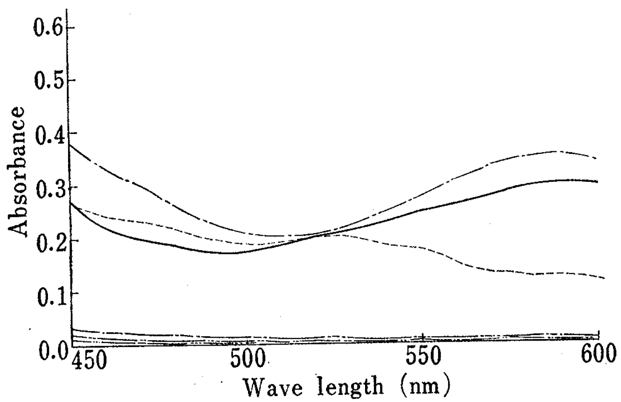

Fig. 5. Visible Absorption Spectra of the Colored Solution produced from Various Sugars with H_2SO_4 and Ethylmercaptan

——————: glucose (100 μg)
- - - - - -: fructose (100 μg)
——·——: galactose (100 μg)
——··——: xylose (100 μg)
—·—·—: ribose (100 μg)
——···——: arabinose (100 μg)

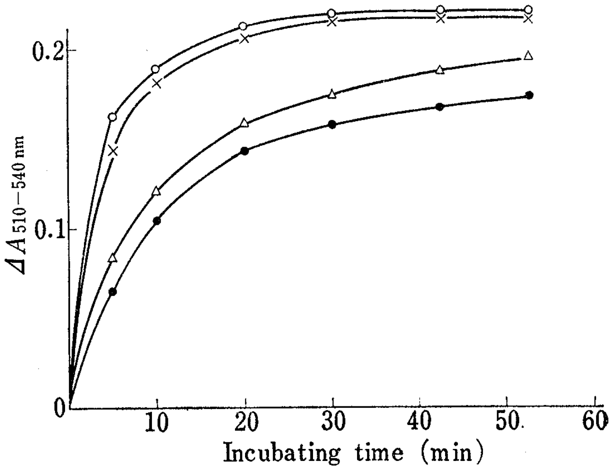

Fig. 6. Effects of Incubating Time and Temperature upon Color Development of Sedoheptulosan with Thioglycolic Acid

—△—: 0.97 mg (8.5 μmoles) thioglycolic acid, at 20°
—●—: 3.88 mg (34.0 μmoles) thioglycolic acid, at 20°
—×—: 0.97 mg (8.5 μmoles) thioglycolic acid, at 50°
—○—: 3.88 mg (34.0 μmoles) thioglycolic acid, at 50°

in modified sedoheptulosan determination. It is obvious that maximum $\Delta A_{510-540\,\mathrm{nm}}$ was achieved with 34 μmoles of thioglycolic acid at 50° in 30 minutes. When 15 μg of sedoheptulosan was incubated with sulfuric acid and 34 μmoles of thioglycolic acid at 25°, 40°, 50°, and 60° for 30 minutes, mean values of triplicate determinations of $\Delta A_{510-540\,\mathrm{nm}}$ were 0.221, 0.273, 0.317, and 0.302, respectively. $\Delta A_{510-540\,\mathrm{nm}}$ was not greatly changed when 8.5 μmoles of thioglycolic acid was used at 50°. It was, however, apparently affected by the amount of thioglycolic acid at 20° and the difference of absorbancy with 8.5 μmoles of thioglycolic acid was always larger than that with 34 μmoles of thioglycolic acid. The calibration curve for the determination of sedoheptulosan by the modified procedure is shown in Fig. 7. Linear proportionality between $\Delta A_{510-540\,\mathrm{nm}}$ and the concentration of heptosan was obtained at 1—10 μg of the standard solution. The linear relations was in fact held up to 30 μg of sedoheptulosan but the absorbancy at 510 nm with 30 μg of heptosan was approximately 1.20 and the readings would be inaccurate. The preparation of test solution containing 1—10 μg of sedoheptulosan in 0.5 ml should thus be recommended.

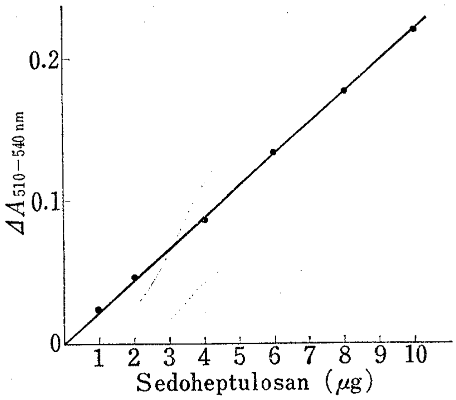

Fig. 7. Calibration Curve of Sedoheptulosan by Modified Dishe's Method

Discussion

It has been shown in Fig. 1 and 6 that the reaction time could be shortened up to 30 minutes with the use of several SH containing reagents at 50° in sedoheptulosan determination. Thioglycolic acid was most suitable among them, especially when pentoses were present (Fig. 3). In the determination of the activity of transketolase, ribose-5-phosphate and its isomerized substances are used as donor and acceptor pentoses. The yield of sedoheptulose in transketolase reaction ranges from few percent to 64 percent[5] of theoretical value and hence the amounts of pentoses, not utilized as substrates would give considerable influence upon sedoheptulose determination. When 10 μg of sedoheptulosan are determined in the presence of 100 μg of xylose according to Dische's and modified method, $\Delta A_{510-540\ \mathrm{nm}}$ should be 0.200 and 0.231. They are 90.5% and 104.5% of real value, respectively. The interferences of phosphorylated sugars which are real substrates, on sedoheptulose determination are in study. Pentose, hexose and heptulose are all reactive with sulfuric acid to give furfural derivatives which then react with SH containing reagents producing colored substances. Their structures are still unknown. It is interesting to note, however, that SH compounds give intense colours for pentoses in the order of their molecular weight; glutathione$>$cysteine$>$thioglycolic acid$>$$H_2S$.

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Tetrahydroauroglaucin from *Penicillium charlesii*

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(Received July 7, 1972)

In our course of chemical examinations on the metabolites produced by toxic fungi,[2] we encountered a strain of *Penicillium charlesii* G. Smith highly colored in the mycelium. Although the fungus had been known to metabolize tetronic acid derivatives,[3] pigment formation had not been reported. Thus chromatographic separation of the chloroform extract of the mycelium was performed to give three pigments; physcion (parietin) (I), flavoglaucin (II), and a new yellow pigment of mp 60—61° (III). The two (I and II) have been known as common metabolites of *Aspergillus glaucus* group and I has also been isolated from some other fungi.[3]

The new pigment (III) has a molecular formula, $C_{19}H_{26}O_3$ (by a high resolution mass spectrum), and shows quite similar spectral properties to II; the infrared (IR) spectrum ($\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3250, 2900, 1620, 1580, 1440, 1260) shows the presence of hydroxyl and hydrogen-bonded conjugated carbonyl groups and the ultraviolet (UV) spectrum ($\lambda_{\max}^{\mathrm{EtOH}}$ nm(log ε): 277, 400 (3.93, 3.69)) is nearly the same but shows a slight bathochromic shift comparing to that of II ($\lambda_{\max}^{\mathrm{EtOH}}$ nm(log ε): 270, 394 (3.85, 3.66)). These spectral data, the molecular formula, and coexistence with II suggested that III might be a dehydro derivative of II, *i.e.* a tetrahydro derivative of auroglaucin[3] (IV), a congener of II in *Aspergillus glaucus* group. The comparison of th nuclear magnetic resonance (NMR) spectra of II and III disclosed clearly the assumption: The signals for the dimethylallyl, bonded and non-bonded hydroxyls, ring hydrogen, and formyl groups are seen in the spectra of both compounds but the methylene signal (δ *ca.* 1.4) corresponding to five methylenes in II was reduced to that of three in III,

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[1] Location: *Kamiyoga-1-chome, Setagaya-ku, Tokyo.*

[2] The work is in progress with the colaboration with Prof. M. Saito, Institute of Medical Sciences, University of Tokyo, and Dr. H. Kurata, this Institute, and their colaborators; *cf.* M. Saito, M. Umeda, K. Otsubo, H. Kurata, S. Udagawa, and S. Natori, *Proc. Japan Cancer Assoc.*, 27th Annual Meeting, Tokyo, 1968, p. 59.

[3] S. Shibata, S. Natori, and S. Udagawa, "List of Fungal Products," University of Tokyo Press, Tokyo, 1964; W.B. Turner, "Fungal Metabolites," Academic Press, London and New York, 1971.