and relative TLC-mobility in appropriate solvent systems. Spot A was determined to contain cholic acid only, while Spot C was the mixture of two dihydroxy bile acids, chenodeoxycholic and hyodeoxycholic acids. These bile acids are the normal constituents of the rat bile.5)

Spot B after hydrolysis was separated regularly into two fractions, each having a TLC-mobility different from those of cholic and hyocholic acids, when submitted to TLC using either of System 7 and 8 of Eneroth,6a) or System A of Siegfried, *et al.*6c) Comparative study of sulfuric acid absorption spectrum indicated that either of the two fractions was clearly distinct from cholic acid and similar to hyocholic acid. However, hyocholic acid is the species-specific bile acid present only in the pig bile.5) Hsia, *et al.*11) have described that β-muricholic acid shows a similar absorption spectrum to that of hyocholic acid. A major one of the two fractions was identical with β-muricholic acid which was isolated from the urine of surgically jaundiced rats receiving hyodeoxycholic acid according to the method of Matschiner, *et al.*12) The minor one was assumed to be α-muricholic acid from the TLC-behavior and the absorption spectrum, although the reference compound was unavailable.

The TLC-procedure described in the present paper is simple, rapid and accurate for separation of the taurine-conjugated trihydroxy bile acids in the rat bile and useful in studying the cholesterol and bile acid metabolism in rats.

Acknowledgement The authors are very grateful to Dr. S. Ikawa, the director of Scientific Relations Department of Taisho Pharmaceutical Co., Ltd., for his continuous encouragement. Thanks are also due to Miss. M. Masaka and Mr. I. Ushioda for their technical assistance.

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[Chem. Pharm. Bull.
20(12)2721—2723(1972)]

UDC 547.313.2.03.04 : 547.979.733.03.04 : 543.422

On the Electronic Interaction of Cobalt Tetraphenylporphine with Tetracyanoethylene as Investigated by ESR

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(Received June 21, 1972)

We have previously2) investigated the reaction of synthetic cobalt tetraphenylporphine (Co-TPP) with molecular oxygen by electron spin resonance (ESR). As a continuation and extension of the work, the electronic interaction of Co-TPP with tetracyanoethylene (TCNE) was now investigated with special interest in π-bonding nature of TCNE. The latter is well-known to be a strong π-acid forming a number of adducts with metal complexes.3–5)

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1) Location: *Bunkyo-ku, Hongo 7-3-1, Tokyo.*
2) K. Yamamoto and T. Kwan, *J. Catalysis*, **18**, 354 (1970).
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The ESR spectrum of Co(II)-TPP dissolved in chloroform–pyridine appeared to be characterized with 3 *shf* lines at 77°K even in the presence of excessive amounts of pyridine (Fig. 1). The fact may indicate that only one pyridine molecule ($I=1$ for ^{14}N) combines to cobalt atom perhaps along with the z axis to form a five-coordinated complex. In chloroform–tetrahydrofuran (THF), the complex gave a similar spectrum but lacking *shfs*, suggesting that a five-coordinated complex have been formed also in this solution involving THF as the axial ligand.

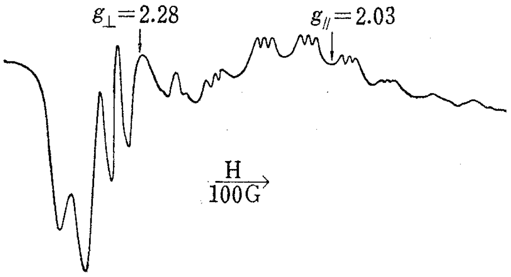


Fig. 1

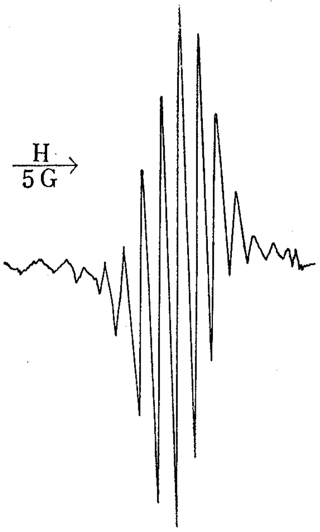


Fig. 2

On adding threefold excess of TCNE to the chloroform–THF solution of Co-TPP, the ESR spectrum due to Co(II) was completely disappeared while an isotropic spectrum with 9 *hf* lines was newly observed at room temperature (Fig. 2). This spectral change should be associated with the electron transfer from cobalt to TCNE, because the newly generated spectrum ($g=2.0026$, $A_N=1.58$ G) is typical of TCNE anion radical.⁶⁾ Absence of splitting due to cobalt nucleus however suggests that the charge transfer from Co(II) to TCNE is extensive so as to release free TCNE anion radicals in the solution.

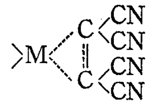
The reaction of Co-TPP with an equal amount of TCNE in chloroform–THF followed by evaporation gave a solid material, which may be formulated as Co-TPP (THF) (TCNE) on the basis of the elementary analysis:

Calcd. for $C_{54}H_{36}ON_8Co$; C, 74.38; H, 4.16; N, 12.86.

Found: C, 74.52; H, 3.88; N, 12.47.

This solid complex gave rise to a single resonance line ($g\sim2$, $\varDelta H_{msl}\sim8$ G) at room temperature, but when dissolved in chloroform–THF, it gave an ESR spectrum similar to the one shown in Fig. 2.

The infrared absorption spectrum (KBr) of the solid complex showed a single C≡N stretching frequency at 2200 cm^{-1}. This value is somewhat lower than that of uncomplexed TCNE (2228 and 2260 cm^{-1}).⁷⁾ Rettig and Wing,⁵⁾ studying infrared spectra of adducts of TCNE with certain organometallic compounds of Pt, Pd, Rh, Cr, and Ir, have already pointed out that most TCNE complexes reveal a single C≡N stretching frequency near 2220 cm^{-1} and that the exhibition of this single C≡N vibration is diagnostic of the structure represented by



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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]

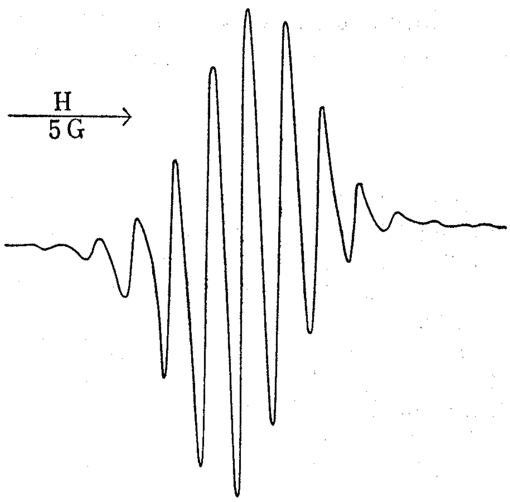

Fig. 3

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Chem. Pharm. Bull.
20(12)2723—2727(1972)

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).

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