TABLE II

Representative quaternary separations of alkaloids on hydrous zirconium oxide paper

Alkaloid separated (R_f values in brackets)	Eluent
Codeine (69)-Morphine (85)-Thebaine Narcotine (53)	*a*
Codeine (70)-Papaverine (85)-Thebaine (94)-Narcotine (53)	*a*
Strychnine (76)-Narcotine (54)-Quinine (62)-Brucine (88)	*a*
Codeine (92)-Morphine (00)-Thebaine (53)-Strychnine (75)	*b*
Codeine (90)-Narcotine (00)-Thebaine (53)-Strychnine (76)	*b*
Morphine (00)-Papaverine (54)-Strychnine (76)-Atropine (96)	*b*

$a = 10^{-3}$ N HCl; $b = 10^{-3}$ N NaOH.

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REINECKE-ACID ANALOGUES WITH 2-SUBSTITUTED BENZIMIDAZOLES

IN one of our previous communications[1] we have reported that benzimidazole (LH = BzH) forms the Reinecke-type acid, $H[Cr(NCS)_4(LH)_2]$, whereas 2-methyl benzimidazole (LH = MeBzH) forms the cationic-anionic complex, $[Cr(NCS)_2(LH)_4][Cr(NCS)_4(LH)_2]$, besides the Reinecke-acid analogue, by substitution in $K_3[Cr(NCS)_6]$. Here we report a more detailed study about the effect of alkyl or aryl substitution at 2-position in benzimidazole on thiocyanate replacement in $Cr(NCS)_6^{3-}$.

2-Ethyl- and 2-propyl-benzimidazoles have been found in the present investigation to form the acids, $H[Cr(NCS)_4(LH)_2]$ only and not the cationic-anionic complexes, presumably on account of the increased steric hinderance. The complexes were obtained as rose-red solids by refluxing the ligand with $K_3[Cr(NCS)_6]$ in 2 : 1 molar ratio in 95% ethanol and diluting the resulting mixture with a large volume of water. The compounds were purified by repeated reprecipitation from ethanolic solution and dried over $CaCl_2$ (Analyses: (i) Found: Cr, 8·97; C, 46·35; H, 3·50; N, 19·76; Required for $H[Cr(NCS)_4(EtBzH)_2]$ (I): Cr, 9·02; C, 45·75; H, 3·65; N, 19·44%. (ii) Found: Cr, 8·72, C, 47·95; H, 4·90; N, 20·12; Required for $H[Cr(NCS)_4(PrBzH)_2]$ (II), Cr, 8·74; C, 48·48; H, 4·12; N, 19·79%).

The molar conductance values (in ethanol at 302° K) of 42·3 and 57·0 ohm^{-1} cm^2 for the ethyl and the propyl derivatives, respectively, fall short of the values expected for monobasic acids. This may be on account of low mobilities of the large anions and strong ion-pair formation between H^+ and the complex anions in ethanol.

The room-temperature (302° K) magnetic moment of 3·83 BM for the ethyl derivative is characteristic of Cr(III) in octahedral site symmetry. The magnetic measurement of the other compound, however, could not be made on account of low yield.

In both these complexes the thiocyanate groups appear to be N-bonded as suggested[3] by the appearance of very strong CN stretching bands near 2120 cm^{-1} (2120 in I and 2118 in II), very weak CS stretching bands near 800 cm^{-1} (803 for I and 798 for II) and NCS deformation modes near 490 cm^{-1} (485 for I and 490 for II). It may be mentioned here that M–NCS bonding in the case of BzH- and MeBzH-complexes were suggested by Ghosh and Mishra[1] mainly on the basis of ν (CS) appearing at 775–785 cm^{-1}. In the present investigation, however, we have confirmed their idea by locating strong bands at 490 cm^{-1} in BzH-complex and at 495 cm^{-1} in MeBzH-complex on account of NCS deformation.

The electronic spectra reveal that the ethyl and the propyl benzimidazole complexes have the same 10 Dq values, *i.e.*, 17,700 cm^{-1}, as methyl benzimidazole complex has, but lower than that of BzH-complex (18,520 cm^{-1}). These values have been calculated from the transition $^4A_{2g} \rightarrow {}^4T_{2g}$. The second ligand-field band on account of $^4A_{2g} \rightarrow {}^4T_{1g}$ (F) transition appears at 24,270 cm^{-1} in EtBzH-derivative and at 25,000 cm^{-1} in PrBzH complex. The two ligand-field bands enable us to calculate the interelectronic repulsion parameter B (588 cm^{-1} for I and 631 cm^{-1} for II) and nephelauxetic ratio β_{35} (0·63 and 0·68, respectively) which are similar to or a bit higher than those of BzH- and MeBzH-complexes.

An aryl (*e.g.*, phenyl) substitution in benzimidazole, appears to have reduced the coordinating capacity

of the ligand to an extent that the ligand forms a benzimidazolium salt with $Cr(NCS)_6^{3-}$ instead of entering the coordination zone.

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DEHYDROASCORBIC ACID REDUCTION IN GUINEA PIG TISSUES

DEHYDROASCORBIC ACID (DHA) is reduced nonspecifically by sulfhydryl compounds like reduced glutathione (GSH), cysteine or homocysteine. Hughes[1,2] suspected the presence of a GSH dependent DHA reductase in the guinea pig liver. However, we observed that DHA reduction in the guinea pig tissue was not enzymic but chemical and the reduction was carried out by DTNB [5-5′-dithio bis-(2-nitrobenzoic acid] reacting thiol compounds, particularly GSH. The reduction was markedly enhanced when instead of GSH, a GSH regenerating system was used.

Soluble supernatant was prepared from 20% tissue homogenates (liver, kidney and pancreas; 10% for adrenal) in isotonic Sörensen buffer, pH 7·0, by centrifuging at 105,000 × g for 1 hour.

DHA was prepared freshly before use by the oxidation of AA with recrystallised *p*-benzoquinone[3]. The incubation medium contained 0·2 ml of tissue soluble supernatant, 33·4 μ moles of Sörensen buffer, pH 7·0, in a total volume of 0·9 ml. In experiments done with GSH alone, the soluble supernatant was replaced by an equivalent amount of GSH solution. The GSH regenerating system contained, in addition to the soluble supernatant and buffer, 1 μ mole G6P (glucose-6-phosphate), 0·025 μ mole NADP, 0·25 μ mole oxidised glutathione (GSSG) and 0·01 μ mole FAD. After preincubation at 37° C for 10 minutes, 0·1 ml of DHA solution (2·84 μ moles) was added and the mixture was incubated at 37° C for 5 to 30 minutes. Incubation was stopped by adding 1 ml of 10% metaphosphoric acid (HPO_3) solution. Ascorbic acid (AA) formed was estimated in the HPO_3 extract by titration with 2, 6-dichlorophenol indophenol. AA was identified by thin layer chromatography ; Rf 0·2 and 0·5 using phenol : water (5 : 1, v/v) and phenol : water : formic acid (10 : 2 : 1, v/v) respectively.

Sulfhydryl (–SH) content was estimated as GSH by the DTNB method of Beutler[4]. GR (glutathione reductase, EC 1·6·4·2) and G6PD (glucose-6-phosphate dehydrogenase, EC 1·1·1·49) were assayed by the method described in Richterich[5]. GSSG was identified by the fall of absorption at 340 nm after addition of NADPH (0·05 μ mole) and GR (0·05 unit, Sigma G 6004) to 1 ml of the incubated mixture. The incubation mixture contained 2·84 μ moles DHA, 1 μ mole GSH and 16·7 μ moles Sörensen buffer, pH 7·5; incubated for 30 min at 37° C. The products of incubation of DHA with GSH were AA and GSSG.

The DHA reducing capacity of the tissue homogenate expressed in μ moles AA formed per ml was recovered in the soluble supernatant. The reducing capacity of the soluble supernatant was dependent on the –SH content. The –SH free dialysed soluble supernatant was incapable of DHA reduction; the activity could be regained by addition of an equivalent amount of GSH. The –SH contents of different tissues expressed in terms of mg GSH/g protein were liver, 10·94, pancreas 7·41, adrenal, 4·48 and kidney, 4·04 respectively. The DHA reducing capacity of the tissues expressed in terms of μ moles of AA formed/g protein/30 minutes at 37° C was liver, 32·14, pancreas, 26·76, adrenal, 17·58 and kidney, 16·75 respectively. The soluble supernatant could be replaced by a protein free boiled extract of the soluble supernatant (10 min. at 98° C) or by an equivalent amount of GSH. When GSH was replaced by a GSH regenerating system consisting of soluble supernatant, G6P, NADP, GSSG and FAD, the rate of DHA reduction was markedly enhanced (Fig. 1). In this system, the rate of DHA reduction was dependent on both the G6PD and GR activities of the soluble supernatant. The G6PD activities of the soluble supernatant of liver, pancreas, kidney and adrenal were 16·35, 66·98, 38·42 and 100·50 I.U./g protein respectively; the corresponding GR activities were 72·66, 130·98, 116·12 and 44·65 I.U./g protein respectively. The results given in Fig. 1 indicate that in the guinea pig tissues, DHA reduction is coupled with the GSH regenerating system as shown in Scheme 1. The oxidation of AA to DHA takes place probably in the mitochondria via cyt c[6]. In the GSH regenerating system, the tissue soluble supernatant could be replaced by an equivalent amount of G6PD (Sigma G 1878) and GR (Sigma G 6004). Dehydroepiandrosterone (4×10^{-5} M), which inhibited G6PD activity of tissue soluble supernatant by 80%, inhibited the DHA reduction by about 80%.

The validity of Scheme I was also supported by the fact that in the –SH free liver soluble supernatant the rate of reduction of DHA was dependent on the concentrations of GSSG and NADP. In comparison with