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Partition coefficients of the iron (III) complexes of pyridoxal isonicotinoyl hydrazone and its analogs and the correlation to iron chelation efficacy. Correction of some reported partition coefficients

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Pyridoxal isonicotinoyl hydrazone (PIH), salicylaldehyde benzoyl hydrazone (SBH), and their analogs chelate iron(III) and show promise as orally effective drugs for treating diseases of iron overload. Their biological activity is related to their lipophilicity, as measured by their partition coefficients *P* between n-octanol and water. However, the method of calculating log *P* described in an article in this journal (Edward *et al.* 1995; *BioMetals*, 8, 209–217) is faulty for compounds such as PIH, SBH and their analogs which contain adjacent hydrophilic groups. Consequently, the calculations reported in the article, based on erroneous log *P* values of the chelating molecules, give erroneous log *P* values of the iron(III) complexes. The chelators most effective in mobilizing ^{59}Fe from reticulocytes have log $P \approx 2.8$, not log $P \approx 0$ and the iron(III) complexes of the most effective chelators have log $P \approx 3.1$, not log $P \approx 0$.

Keywords: iron chelation, iron overload disease, pyridoxal isonicotinoyl hydrazone

Ponka and his colleagues (1979a, 1979b, 1985, 1988) found that pyridoxal isonicotinoyl hydrazone (PIH: **1**, R = 4-pyridinyl) and analogs with varying R groups (the 100 series) and salicylaldehyde benzoyl hydrazone (SBH: **2**, R = phenyl) and analogs (the 200 series) chelate iron(III) and show promise as orally effective drugs for iron overload diseases. The structures of **1** and **2** are given in Figure 1. The biological activity of these compounds varies with their lipophilicities, as measured by their partition coefficients *P* between n-octanol and water (Hansch 1969). A paper by Ponka *et al.* (1994) claimed that maximum activity in mobilizing ^{59}Fe from reticulocytes was shown by compounds having $P \approx 1$ (log $P \approx 0$).

It seems likely that the activity of iron-chelating compounds in removing iron(III) from reticulocytes and other cells will depend not only on the partition coefficient P_{chel} of the chelating agent, which determines the rate of transfer of its molecules across the cell membrane and into the cell, but also on the partition coefficient P_{comp} of the iron–chelate complex formed within the cell, which determines the rate egress of the complex across the membrane and out of the cell. Porter *et al.* (1988) found that *N*-alkyl-3-hydroxypyridin-4-ones, which coordinate iron(III) in a ratio of 3:1 to form octahedral complexes, show maximum activity in removing iron(III) from hepatocytes when log $P_{chel} \approx 0$. According to the equation:

$$\log P_{comp} = n \log P_{chel} + k \qquad (1)$$

(with $n = 3.34$ and $k = -0.61$ for *N*-alkyl-3-hydroxypyridin-4-ones; see Edward *et al.* 1995), the most active chelators will form complexes having log $P_{comp} \cong -0.6$; i.e. both log P_{chel} and log P_{comp} will be close to zero.

Similar arguments were advanced (Ponka *et al.* 1994, Edward *et al.* 1995) to support the claim that compounds of the 100 and 200 series also show maximum activity in mobilizing iron(III) from

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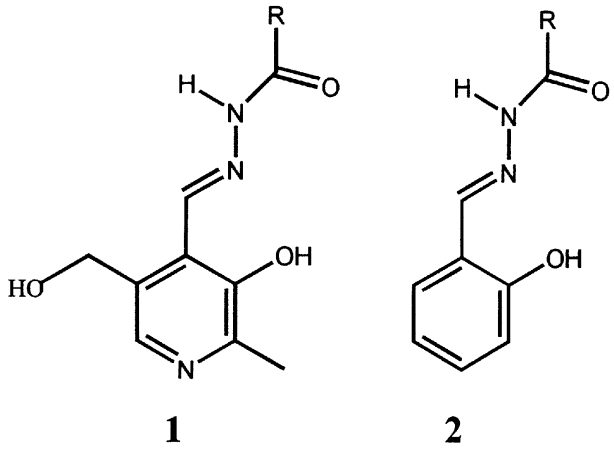

Figure 1. Structures of pyridoxal isonicotinoyl hydrazone (PIH: **1**, R = 4-pyridinyl; compound number 111 of the 100 series) and salicylaldehyde benzoyl hydrazone (SBH: **2**, R = phenyl; compound number 201 of the 200 series). For structures and numbers of compounds see Edward *et al.* (1995).

reticulocytes when both log P_{chel} and log P_{comp} approximate zero, suggesting that biological activity depends primarily on the partition coefficient rather than on the particular structure of the chelating compound (Edward *et al.* 1995).

These arguments are fallacious, being based on erroneous partition coefficients. The log *P* values used in Ponka *et al.* (1994) and Edward *et al.* (1995) were calculated by an additive scheme due to Rekker (1977) (described in essentially identical words in both papers) which gives satisfactory results when hydrophilic groups are sufficiently separated on the molecular framework, but erroneous results when they are adjacent (as the groups –CH=N– and –NH–CO– are in **1** and **2**: Edward *et al*. 1997). In fact, experiment shows that the log P_{calc} values reported for compounds of the 100 series are on average too low by 3.0 units, and for compounds of the 200 series too low by 2.1 units. Consequently, chelating compounds of the 100 and 200 series with maximum activity in mobilizing iron(III) from reticulocytes have log *P* values clustering about 2.8, not zero (Edward *et al.* 1997).

The argument that the complexes of the most active chelators also have log $P_{comp} \approx 0$ is also false. The argument depended first on deriving log P_{comp} from log P_{chel} by application of equation 1, after *n* and *k* for this equation have been established. This was done successfully, but by working in the partitioning system ethyl acetate–water rather than in the standard system, n-octanol–water. For 13 compounds of the 100 series and their 13 iron(III) complexes, log P_{chel} and log P_{comp} were measured, and found to obey equation 1 reasonably well ($r = 0.92$), with $n = 1.94$ (theoretical $n = 2$) and $k = -0.83$. There is no reason to question this experimental work. However, the next step, the calculation of the n-octanol–water log *P* from log P^{EtAc} by application of Collander's equation (1951):

$$\log P^{EtAc} = a \log P + b \qquad (2)$$

was faulty because of the use of erroneous log *P* values gave incorrect values for the constants *a* (0.47) and *b* (1.08) in this equation, which were then used in subsequent calculations. Using the experimentally determined values log *P* for twenty chelating compounds (Edward *et al.* 1997), a reasonably linear relationship between log P^{EtAc} and log *P* is found ($n = 18$, $r = 0.92$), as shown in Figure 2, (omitting points for compounds 106 and 202 as outliers) and we now obtain slope $a = 0.69$ and intercept $b = -0.81$. These values of *a* and *b* are slightly closer to the values reported by Leo & Hansch (1971) ($a = 0.93$, $b = 0.05$) on the basis of log *P* and log P^{EtAc} values for nine solute molecules (four monocarboxylic acids, two dicarboxylic acids, and the polyfunctional compounds dipterex, chloramphenicol and benzylpenicillin), but the differences are substantial: they point out the limitations of Collander's equation. These limitations have already been noted by Leo & Hansch (1971), who found systematic differences in *a* and *b* when the equation was applied to solute molecules having systematic differences in numbers of hydrogen-bonding sites.

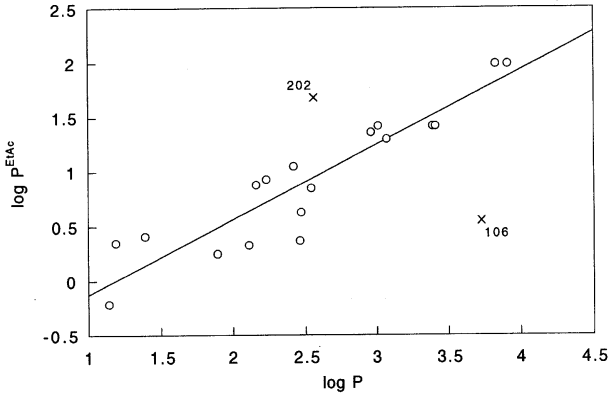

Figure 2. Plot of experimental log P_{chel} (ethyl acetate–PBS) values of twenty chelating compounds of the 100 and 200 series (Edward *et al.* 1995) against log P_{chel} (n-octanol–water) values (Edward *et al.* 1997). The points for compounds 106 and 202 are considered outliers, and are disregarded in applying regression analysis to fit the straight line to the other 18 points of this plot.

Chelating compounds showing maximum biological activity when they have log $P_{chel} = 2.8$ (see above) will have log $P^{EtAt}_{chel} = 1.1$ (from equation 2) and form iron(III) complexes having log $P^{EtAc}_{comp} = 1.3$ (equation 1, with $n = 1.98$ and $k = -0.83$; Edward *et al.* 1995) and thence log $P_{comp} = 3.1$ (equation 2), not 0. This value is likely to be only approximate.

In summary, the derivation of equation 1 in Edward *et al.* (1995) is sound, but the method described for calculating log *P* is faulty when applied to compounds of the type considered in the paper, so that the log *P* values of the chelating compounds, the values of *a* and *b* in Collander's equation, and the log *P* values calculated for iron(III) complexes using this equation are all seriously in error.

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