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Ionisation constants and partition coefficients of some analgesically active 2-benzylbenzimidazole derivatives and related compounds

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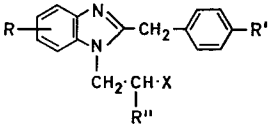
The ionisation constants (in aqueous ethanol) and the partition coefficients (in a water-cyclohexane system) of some analgesically active 2-benzylbenzimidazole derivatives and related compounds are reported. The results are discussed in terms of structure-activity relationships.

IN 1957 a novel class of analgesics (I), related to 2-benzylbenzimidazole, was reported (Hunger, Kebrle, Rossi & Hoffman, 1957, 1960). Certain of these compounds were highly active, their potencies in animal tests far exceeding that of morphine and of any synthetic analgesic known at that time** (e.g. Ia-d). The action of these derivatives appears to be typically morphine-like. Thus, their side-effects in man include respiratory depression and constipation (Bromig, 1958), while, in addicts, the two derivatives (Ia and f) have addiction potentials comparable with that of morphine (Fraser, Isbell & Wolbach, 1960).



	R	R'	Activity† (morphine = 1)
a	5-NO_2	OEt	1000
b	5-NO_2	OisoPr	500
c	5-NO_2	OMe	100
d	H	OEt	70
e	H	OMe	1
f	5-NO_2	Cl	3
g	6-NO_2	Cl	0·1

† in mice, tail pressure method (Hunger & others, 1960).

In common with other classes, analgesics based upon 2-benzylbenzimidazole possess a flat aromatic system linked to a 2-aminoethyl side-chain via a non-hydrogen bearing atom [features considered essential for fit at the proposed analgesic receptor site (Beckett & Casy, 1954, 1965)], their distinctive structural features being as follows: (1) two basic centres (most analgesics are monobasic), (2) a bicyclic aromatic nucleus (most analgesic molecules contain a single benzene ring or two isolated 5-or 6-membered

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** Since 1957 synthetic analgesics of similarly high potencies have been reported, e.g. t-alcohols derived from the thebaine-vinyl methyl ketone adduct (Bentley & Hardy, 1963).

aromatic rings), (3) a 4- alkoxy substituent in the benzyl and a 5-nitro-group in the benzimidazole fragments of the more active derivatives. One or more of these features may be partly responsible for the high potencies observed in these derivatives, either by a direct influence upon the intrinsic activity of the molecule, or by one upon factors involved in the transport of the drug molecule to its locus of action [such transport is considered to be a simple diffusion process which is governed principally by the dissociation constant of the drug and by the lipid solubility of the unionised form of the drug (Brodie & Hogben, 1957; Brodie, Kurz & Shanker 1960)]. The present physico-chemical study was undertaken with a view to making a preliminary assessment of certain structure-activity relationships in 2-benzylbenzimidazole analgesics, with particular reference to the influence of structure upon drug transport.

Experimental

All benzimidazole derivatives used were prepared by reported methods (Hunger & others, 1960; Casy & Wright, 1966). 2-Benzyl-5-nitro-(2-diethylaminoethyl) benzimidazole formed a monohydrochloride monohydrate, m.p. 199–200° (found: C, 58·6; H, 6·75; Cl, 9·05. $C_{20}H_{25}ClN_4O_2 \cdot H_2O$ requires: C, 59·05; H, 6·6; Cl, 8·7%), ν_{max} 3500 cm^{-1} (H_2O) and 1-(2-diethylaminoethyl)-2-(4-ethoxybenzyl)-5-nitrobenzimidazole, a monohydrochloride monohydrate, m.p. 160° (found: C, 57·9; H, 6·8. $C_{22}H_{29}ClN_4O_3 \cdot H_2O$ requires: C, 58·6; H, 6·9%), ν_{max} 3400 cm^{-1} (H_2O), when treated with excess of ethanolic hydrogen chloride.

Ionisation constants. The pK'_a values of acids conjugate to the bases* (the superscript indicates that values are uncorrected for ionic strength) were determined in 50% water (ion-free)-ethanol by the method of Albert & Serjeant (1962) (water could not be used because the bases were sparingly soluble in this solvent). The base hydrochloride (0·25 mmole) was dissolved in the solvent mixture (47·5 ml) and the stirred solution titrated under nitrogen with 0·05N sodium hydroxide (carbonate free) at 25°. The titrant was added in ten equal portions of 0·5 ml and the pH of the mixture recorded after each addition as soon as equilibrium had been reached. A Pye Dynacap pH meter (with a calomel and a screened glass electrode) was used to make these measurements; the glass electrode was standardised by 0·05 M potassium hydrogen phthalate (pH 4·00) and 0·05 M borax (pH 9·15) buffers, and the accuracy of the apparatus and technique were checked by measuring the pK_a of benzoic acid [pK'_a 4·09 in water, Albert & Serjeant (1962) give 4·12]. pK_a values were calculated by applying Henderson's equation to each of the nine pH values obtained during the potentiometric titrations, and averaging the results. Average values were only acceptable if the scatter did not exceed $\pm$ 0·08 pK'_a unit.

Partition coefficients. The partition coefficients of the benzimidazole derivatives were determined between cyclohexane (spectroscopic grade) and an aqueous buffer of pH 7·4, prepared by mixing 0·1 N sodium hydroxide (39·5 ml) with 0·1 M potassium dihydrogen phosphate (50 ml)

* Henceforth for brevity referred to as the pK'_a values of the bases.

and adjusting the volume to 100 ml (Lange, 1952). For the partitioning, cyclohexane saturated with buffer solution and buffer solution saturated with cyclohexane were used. Analysis of the concentrations of partitioned substances were made using a Uvispek H. 700/304 spectrophotometer. The absorption peaks near 248 mμ (for non-nitrobenzimidazoles) and 240 mμ (for nitrobenzimidazoles) were used in these analyses, absorption determinations being made of a suitable dilution of the aqueous layer after partitioning. In the case of morphine, the catechol chromophore (λ_{max} 287 mμ, ϵ 2700) was employed for spectroscopic measurements. The small amount of cyclohexane dissolved in the water had no effect on the absorption curves at wavelengths higher than 220 mμ (in contrast, ethylene dichloride had a marked effect and could not be used as the organic phase on this account). The amount of sample and the volume of the two phases chosen was such that a onefold dilution of the aqueous phase gave an absorbance of between 0·2 and 0·8 using a 1 cm cell. Calibration curves were drawn for each compound studied (Beer's Law was obeyed over this absorbance range in all cases).

Procedure. Ten ml of each liquid phase was pipetted into a Quickfit tube, and after the addition of about 5 mg of the sample (accurately weighed), the tube was sealed and shaken horizontally for 24 hr at 37°. After the two layers had separated, the aqueous phase was run off and a 5 ml pipetted sample diluted to 10 ml with 0·002 N hydrochloric acid before measuring its absorbance. Each determination was made in duplicate.

The ratio C_1/C_2, where C_1 is the solute concentration in cyclohexane and C_2, that in the aqueous buffer, gives the apparent partition coefficient (P') (Reese, Irwin, Dittert, Chong & Swintosky, 1964), since C_2 includes both ionised and unionised forms of the benzimidazole derivatives. The true partition coefficient (P) involves only molecular species common to both phases and is obtained from the expression

$$P = \frac{C_1}{C_2\,(1-\alpha)}$$

where α is the degree of ionisation of the bases at pH 7·4 [α is calculated from the equation

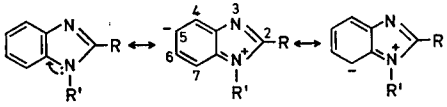
$$\text{Per cent ionised} = \frac{100}{1 + \text{antilog}\,(7{\cdot}4 - pK_a)}$$ (Albert, 1960)].

P values (for unionised base) have been calculated on the assumption that the pK_a values of the bases in water are 0·5 pH units higher than those in 50% ethanol-water*, and that the bases are completely unionised in cyclohexane.

* Although anomalies may arise from the use of mixed solvents in some cases (Albert & Serjeant, 1962), a number of workers (Mizutani, 1925; Hall & Sprinkle, 1932) have found that, in a series of related bases, the use of 50% aqueous ethanol consistently caused a depression of approximately 0·5 units in the pK_a compared to the value obtained in water. This was also found to be so with a series of benzimidazole derivatives studied by Davies, Mamalis, Petrow & Sturgeon (1951).

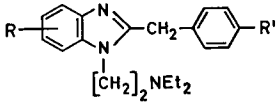
Results

2-Benzylbenzimidazole, the parent molecule of the derivatives studied here, is a weak base, its pK′$_a$ value (5·01) being similar to that of benzimidazole itself [4·98 in 50% aqueous ethanol (Rabiger & Joullié, 1964); 5·53 in water (Schwarzenbach & Lutz, 1940)]. Corresponding 5(6)- and 4(7)-nitro-derivatives have pK′$_a$ values approximately two units lower than that of the unsubstituted molecule (e.g. Table 1, Nos 2–5), the base-weakening influence of the nitro-group probably being a result of its stabilising resonance forms such as (II and III) with a consequent drift



II III

TABLE 1. IONISATION CONSTANTS[1] AND PARTITION COEFFICIENTS[2] OF SOME 2-BENZYLBENZIMIDAZOLE DERIVATIVES



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No	R	R′	R″	X	pK′a		% ionised at pH 7·4[7]	Partition coefficient	
					Side-chain nitrogen	Hetero-cyclic nitrogen		Apparent (P′)	Corrected (P)
1[3]	H	H	—	—	—	5·01[4]	—	—	—
2[3]	5(6)-NO_2	H	—	—	—	2·94	—	—	—
3[3]	4(7)-NO_2	H	—	—	—	2·84	—	—	—
4	5-NO_2	H	H	OH	—	2·84	—	—	—
5	5-NO_2	H	Me	OH	—	3·10	—	—	—
6	H	H	H	NMe_2	6·49	4·12	29	4·3	6
7	H	H	Me	NMe_2	6·51	[5]	—	—	—
8	H	OEt	Me	NMe_2	6·80	4·05	44	11	20
9	H	OEt	Me	NEt_2	6·90	4·10	50	15	30
10	5-NO_2	H	H	NEt_2	6·34	2·83[6]	22	57	73
11	6-NO_2	H	H	NEt_2	6·35	3·71	22	89	114
12	5-NO_2	H	Me	NEt_2	6·70	3·07	—	—	—
13	5-NO_2	OEt	H	NEt_2	6·36	2·86[6]	22	76	97
14	5-NO_2	OEt	Me	NEt_2	6·67	3·03	40	78	130

[1] in 50% aqueous ethanol at 25°
[2] in an aqueous buffer (pH 7·4)—cyclohexane system
[3] N(1) side-chain absent
[4] Rabiger & Joullié (1964) give pKa 5·7 in 95% aqueous ethanol
[5] not measured
[6] pKa measured by adding excess of acid and back titrating (dihydrochloride could not be isolated)
[7] calculated from the expression

$$\% \text{ ionised} = \frac{100}{1 + \text{antilog}\,[7{\cdot}4 - \text{pK}_a\,(H_2O)]} \text{ (Albert, 1960);}$$

it is assumed that pKa (H_2O) = pKa (50% aqueous-ethanol) + 0·5.

of electrons away from the basic centre. 1-Dialkylaminoethyl-2-benzylbenzimidazoles have two basic centres, that of the side-chain nitrogen being the stronger by a factor of almost 10^3 (e.g. Table 1, Nos 8 and 9). The heterocyclic basic centre (pK'_a near 4) in such derivatives is approximately ten times weaker than that of 2-benzylbenzimidazole, proton uptake at this centre being opposed by a positive charge on the side-chain nitrogen. [Nuclear magnetic resonance and ultraviolet spectral evidence for N(1) rather than N(3), being the site of protonation of the 1-substituted benzimidazole derivatives will be presented elsewhere.] In 1-(2-dialklyaminoethyl)-2-benzylnitrobenzimidazoles, the nitro-substituent lowers the pK'_a of the heterocyclic centre to values similar to those of the nitro-derivatives discussed previously, but has little influence upon the pK'_a of the side-chain nitrogen atom (Table 1, Nos 10, 12–14). When the nitro-group is in the 6-position (Table 1, No. 11) the pK'_a of the heterocyclic nitrogen atom is 0·7–0·9 units higher than that of corresponding 5-nitro-derivatives (Table 1, No 10, 12–14). This result is interpreted in terms of the 6-nitro-group being less effective than a 5-nitro-group in stabilising resonance contributors such as (II and III) because resonance interactions between a 6-nitro-group and a negative charge at C(5) or (7) are not possible; hence the electron withdrawing influence of the nitro-group may only operate by the less effective inductive mechanism. As a result, its base-weakening influence is less than that of a 5-nitro-group, the pK'_a of the heterocyclic nitrogen in the derivative (Table 1, No 11) being close to those of analogues lacking a nitro-group (Table 1, Nos 6, 8 and 9). A 2-methyl substituent in the aminoethyl side-chain appears to have a small base-strengthening effect upon both centres (cf. Table 1, Nos 10 and 12; 13 and 14), and it may be of significance, in this respect, that while the 2-methylethyl derivatives (Table 1, Nos 12 and 14) formed dihydrochlorides when treated with excess of ethanolic hydrogen chloride, the unbranched analogues (Table 1, Nos 10 and 13) formed monohydrochlorides under the same conditions.

In the partition work, the distributions of benzimidazole derivatives between an aqueous buffer at pH 7·4 (close to physiological pH) and cyclohexane (an organic solvent intended to simulate a lipid membrane) were determined. The apparent partition coefficients (P′) and those corrected for ionisation (P) are given in Table 1; the latter values are larger because all the derivatives are partially ionised at pH 7·4. The derivative (Table 1, No. 6), which lacks both nitro- and ethoxy groups, has the lowest partition coefficient of the series. An ethoxy group increases the affinity of the molecule for the organic phase (cf. Table 1, Nos 6 and 8), as does also an increase in size of the side-chain basic group (cf. Table 1, Nos 8 and 9). A 5-nitro-group enhances lipid solubility to a pronounced degree while the effects of nitro- and ethoxy groups appear to be additive in this respect (Table 1, Nos 13 and 14). The influence of a 6-nitro-group outweighs the combined effects of 5-nitro- and ethoxy, the derivative (Table 1, No. 11) having the highest partition coefficient of all the compounds examined. Morphine, in the same solvent system, had an apparent partition coefficient of 0·15 and a corrected one of 0·8 [assuming a pK'_a of 8·05 (Farmilo, Oestreicher & Levi, 1954) in water].

Discussion

The pK'_a values of the 2-benzylbenzimidazole derivatives of Table 1 (some of which are potent analgesics) in 50% aqueous ethanol, lie in the ranges 6·3–6·9 (side chain nitrogen) and 2·9–4·1 (heterocyclic nitrogen); corresponding values in water are assumed to be approximately 0·5 pK_a units higher (see footnote, p. 679). Farmilo & others (1954) have reported the pK'_a values of several different types of analgesics, most of which had values within the range 7·8–8·9 in water or aqueous ethanol (this range corresponding to the bases being approximately 90% ionised as cations at physiological pH). Hence, although exact comparisons cannot be made, the analgesically active derivatives of Table 1, and probably related active derivatives also, are somewhat less ionised than many other classes of analgesic. This is probably due to the fact that the side-chain nitrogen atom in benzimidazole derivatives is linked to the aromatic nucleus by a second nitrogen atom (of high electronegativity), whereas, in most other classes of analgesic the link is a quaternary carbon atom which has a smaller base-weakening influence. Nevertheless, in view of the high activities observed, the extent to which they are ionised at physiological pH (20–50% as monocations–ionisation as dications is negligible at pH 7·4) appears to be high enough to provide sufficient protonated molecules [considered the active species (Beckett & Casy, 1965)] in the vicinity of the receptor site. Hence the dibasic character and the lower (monocation) pK'_a values of benzimidazole analgesics, compared with those of other classes, is probably not significant in regard to the intrinsic activities of these compounds. However, since the penetration of lipid barriers involves non-ionised, rather than ionised, molecules (Brodie & others 1957, 1960), the reduced extents to which benzimidazole analgesics are ionised at body pH, may have an important influence upon the transport of such molecules to their site of action.

All the benzimidazole derivatives (Table 1) have apparent partition coefficients* in the aqueous buffer (pH 7·4)—cyclohexane system, greater than that of morphine (taken as a typical analgesic molecule since it is a monobase and contains one aromatic ring). Differences are particularly extreme between morphine and 2-benzylbenzimidazole derivatives containing nitro- and nitro-ethoxy substituents. The highly potent derivative (No 13, Table 1) for example, is more than 500 times more soluble than morphine in the organic phase.

Assuming the distribution of a compound in cyclohexane-water (pH 7·4) to reflect its partition between physiological lipid and non-lipid phases, these results suggest that the benzimidazole derivatives (I), especially those containing ethoxy and nitro-substituents, will be rapidly transported across lipid barriers to their site of action, once they have been administered. A drug which penetrates the central nervous system rapidly has a greater

* Apparent, rather than corrected, partition coefficients are considered of more value in assessing the influence of the water-lipid distribution properties of drug molecules upon activity because the former constant takes into account ionisation as well as partition factors.

chance of reaching its site of action than one of similar pharmacology whose rate of penetration is slow, because the former drug is less likely to be metabolised or excreted during the time interval elapsing between drug administration and response. Hence, *other factors being equal*, the more potent of two drugs will be the one which more readily penetrates lipid barriers. For these reasons it may be inferred that the extremely high potencies of 1-(2-diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitro benzimidazole and similar derivatives are related to their probable ease of penetration of the central nervous system.

However, transport considerations alone are inadequate in accounting for the influence of ethoxy and nitro-substituents upon activity in this series. The ethoxy derivative (Id), for example, is 70 times more active in mice than the methoxy analogue (Ie) although the two derivatives probably have similar lipid solubilities, this fact reflecting the well-known structural specificity of the analgesic receptor site. The receptor is also sensitive towards the position of substitution of the lipophilic nitro-group. While a 5-nitro-group allows (and may enhance) drug-receptor interaction, a 6-nitro-group (more effective than 5-nitro in increasing lipid solubilities—cf. Table 1, Nos 10 and 11) impedes the same association as is evident from the relative activities of the derivatives (If and g).

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