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Correlation of partition coefficients in n-heptane-aqueous systems with buccal absorption data for a series of amines and acids

A. H. BECKETT AND A. C. MOFFAT*

From the Department of Pharmacy, Chelsea College (University of London), Manresa Road, London, S.W.3, England

Partition coefficients in n-heptane-0·1N sodium hydroxide or 0·1N hydrochloric acid have been determined for a series of amines and acids; linear relations between chain-lengths and the logs of their partition coefficients were found. The plot of alkyl chain-length against buccal absorption of some amphetamines and fenfluramines, when they were 1% unionized, was linear. There was also a linear relation between the logs of the partition coefficients and buccal absorption of the amines and acids when these were 1 and 10% unionized. Those amines and acids having similar partition coefficients, when equally unionized, were absorbed to the same extent in the buccal test over the pH range 4 to 9. During the test the pH at the surface of the buccal membrane was shown to be the same as that of the solution in the mouth. n-Heptane is considered to be equivalent in solvent properties to the buccal lipid membrane for the compounds used in the present test.

For a variety of drugs, positive correlations have been noted between partition coefficients and (*a*) *in vivo* absorption (Walton, 1935; Mayer, Maichel & Brodie, 1959; Schanker, 1959; and others), (*b*) renal tubular reabsorption (Knoefel, Huang & Jarboe, 1961, 1962; Weiner & Mudge, 1964; Wilkinson, 1966; and others) and (*c*) hypnotic activity (Hansch, Maloney & others, 1968). In none of these cases were the permeability characteristics of the membranes examined, whilst the choice of partition systems was made arbitrarily.

After examining a number of solvent systems, we chose n-heptane-water since this solvent pair has negligible mutual solubility and, in general, n-heptane dissolves unionized but not ionized molecules. It was hoped that this system would give partition coefficients that could be related to previously determined buccal absorption results for both basic and acidic drugs.

A further object was to relate partition coefficients and the buccal absorptions of amines and acids when they were unionized to the same extent and from this to establish whether the pH of the buccal mucosal surface was the same as that of the bulk solution.

EXPERIMENTAL

Partition coefficients

Apparatus. Glass tubes (100 ml capacity), closed at both ends, with central

* Present address: Institute for Lipid Research, Baylor College of Medicine, Texas Medical Center, Houston, Texas, 77025, USA.

sampling ports with glass stoppers were used in a rocking device similar to that of Reese, Irwin & others (1964).

Compounds. The compounds examined included those used previously (Beckett & Moffat, 1968, 1969) and also those amines and acids listed in Tables 1 and 2.

Methods. A sample of organic compound (1 mg) in 25 ml aqueous solution (0·1N hydrochloric acid for acids or 0·1N sodium hydroxide for amines, both saturated with n-heptane) was placed in each tube together with 25 ml n-heptane (saturated with the acid or alkali). The ports were stoppered and the rocking machine run for 16 h at 25° ± 1°. Tubes containing the aqueous solutions of the organic compounds were used as controls.

The final concentration in the aqueous phase was determined by gas-liquid chromatographic analysis of 5 ml aliquots of the aqueous layer (Beckett & Moffat, 1968, 1969; Tables 1 and 2). Duplicate analyses were made and the mean taken.

The partition coefficients (K) were then calculated according to the appropriate relation:

$$K = \frac{\text{(Initial-Final) concentration in the aqueous phase}}{\text{Final concentration in the aqueous phase}}$$ for the amines

$$K = \frac{\sqrt{\text{(Initial-Final) concentration in the aqueous phase}}}{\text{Final concentration in the aqueous phase}}$$ for the acids since they dimerize in the heptane phase (units: $ml^{1/2}\,\mu g^{-1/2}$)

Buccal absorption measurements

Aqueous solutions of the amine hydrochlorides and sodium salts of the acids were used with the method of Beckett & Moffat (1968).

For analysis, the method of Beckett & Moffat (1968) was used, except that the amines were extracted from alkaline solution and the following gas-liquid chromatography conditions used: a 2 m, ¼ inch O.D. glass tube packed with Chromosorb G (acid washed, DMCS treated, 80–100 mesh) coated with 2·0% Apiezon L/5% KOH; nitrogen pressure 20 lb/inch², hydrogen pressure 24 lb/inch², and air pressure 30 lb/inch²; injection block temperature approximately 50° above the oven temperature. The oven temperature and internal standard used for each acid and amine not already reported are summarized in Tables 1 and 2.

Table 1. *Gas-liquid chromatography conditions for the analysis of some amines on a 2% Apiezon L/5% KOH column at* 110°

Amine	Retention time (min)	Internal standard
Amphetamine	3·4	*N*-n-Butylnorfenfluramine
N-Methylamphetamine	4·8	,,
N-Ethylamphetamine	6·5	,,
N-n-Propylamphetamine	11·4	,,
N-n-Butylamphetamine	21·2	,,
Norfenfluramine	2·7	*N*-n-Propylamphetamine
N-Methylnorfenfluramine	3·7	,,
Fenfluramine (*N*-ethylnorfenfluramine)	4·9	,,
N-n-Propylnorfenfluramine	8·4	,,
N-n-Butylnorfenfluramine	15·3	,,

Table 2. *Gas-liquid chromatography conditions for the analysis of some acids on a 2·5% SE-30 column*

Methyl ester of acid	Retention time (min)	Oven temp. (° C)	Methyl ester of internal standard	Retention time (min)
Isovaleric	4·0	40	Hexanoic	18·0
2,6-Dimethylbenzoic	11·0	100	*p*-Toluic	7·6
o-Chlorobenzoic	10·2	100	*p*-Toluic	7·6
m-Chlorobenzoic	9·8	100	*p*-Toluic	7·6
Cinnamic	7·2	125	*p*-Chlorobenzoic	3·9
p-Phenoxyphenylacetic ..	16·6	165	*p*-t-Butylphenylacetic	3·0

RESULTS AND DISCUSSION

Partition coefficients

Partition coefficients and pK_a values for all the amines and carboxylic acids used are shown in Tables 3 and 4.

The values of the partition coefficients for members of both the long-chain fatty acid and *p*-n-alkylphenylacetic acid series increase in a regular geometric manner as the alkyl chain lengths increase (Fig. 1A). This phenomenon is also shown by the amphetatamines and fenfluramines (Fig. 1B). Values of K vary within a group of isomers, e.g. the chlorobenzoic acids: *o*, 0·05; *m*, 0·18; *p*, 0·17 $ml^{1/2}\mu g^{-1/2}$, which is due partly to their differences in water solubility, viz. 1/900; 1/2850; 1/5290 respectively (*Merck Index*, 1960).

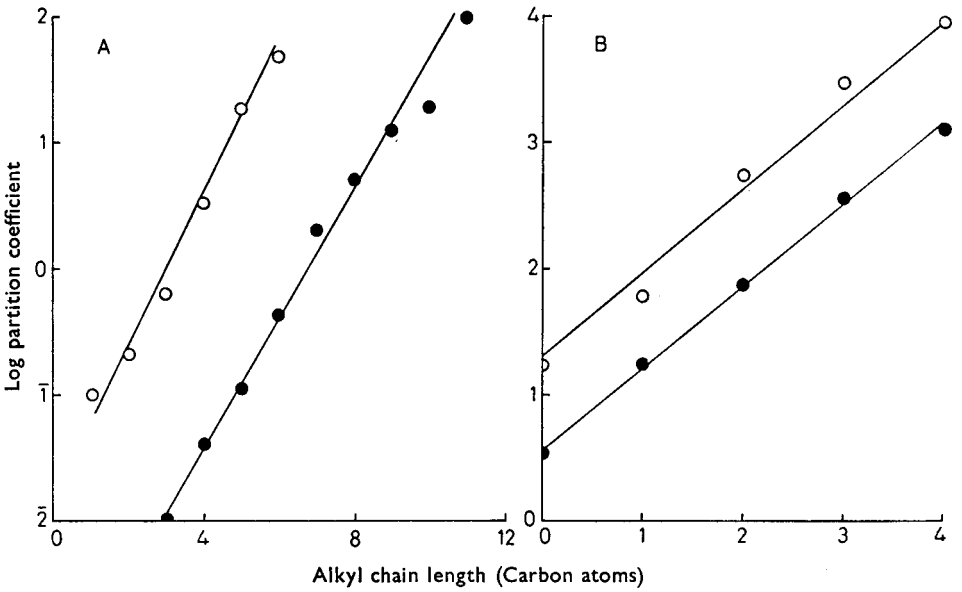

FIG. 1. A. The influence of chain length on the n-heptane-0·1N HCl partition coefficients of two series of acids. ○, *p*-n-alkylphenylacetic acids; ●, straight-chain fatty acids. B. The influence of chain length on the n-heptane-0·1N NaOH partition coefficients of two series of *N*-n-alkyl amines. ○, fenfluramines; ●, amphetamines.

Buccal absorption measurements

The buccal absorptions of the amphetamines and fenfluramines, when 1% unionized, are linearly related to the alkyl chain length. This finding is similar to that for the

Table 3. *n-Heptane-0·1N sodium hydroxide partition coefficients and* pK_a *values for some amines*

Amine	Partition coefficient* 25° C	pK_a 25° C
Amphetamine	3·4	9·70[1]
N-Methylamphetamine	17·3	10·00[1]
N-Ethylamphetamine	75	10·05[1]
N-n-Propylamphetamine	360	10·18[2]
N-n-Butylamphetamine	1270	10·20[2]
Norfenfluramine	17·0	9·53[1]
N-Methylnorfenfluramine	60·3	9·67[1]
Fenfluramine (*N*-ethylnorfenfluramine)	550	9·88[1]
N-n-Propylnorfenfluramine	2800	10·00[3]
N-n-Butylnorfenfluramine	9000	10·00[3]

* Present results, calculated assuming no association of molecules in either phase.

1. Brookes (1968); at 22° C.
2. Present studies.
3. Approximate value, calculated from the amphetamine results.

Table 4. *n-Heptane-0·1N hydrochloric acid partition coefficients and* pK_a *values for some carboxylic acids*

Acid	Partition coefficient[1] 25° C	pK_a 25° C	Acid	Partition coefficient[1] 25° C	pK_a 25° C
n-Butyric	<0·01	4·82[3]	Phenylacetic	<0·01	4·31[3]
iso-Valeric	0·02	4·77[3]	*o*-Tolylacetic	0·04	4·35[4]
n-Valeric	0·04	4·81[3]	*m*-Tolylacetic	0·11	4·36[6]
n-Hexanoic	0·11	4·85[3]	*p*-Tolylacetic	0·10	4·37[4]
n-Heptanoic	0·43	4·89[3]	*p*-Ethylphenylacetic	0·21	4·37[4]
n-Octanoic	1·94	4·85[3]	*p*-n-Propylphenylacetic	0·62	4·36[6]
n-Nonanoic	5·02	4·85[6]	*p*-n-Butylphenylacetic	3·30	4·36[6]
n-Decanoic	12·2	4·85[6]	*p*-t-Butylphenylacetic	3·21	4·36[6]
n-Undecanoic	18·5	4·85[6]	*p*-n-Pentylphenylacetic	18·0	4·36[6]
n-Dodecanoic	95·8	4·85[6]	*p*-t-Pentylphenylacetic	3·4	4·36[6]
Benzoic	0·11	4·20[2]	*p*-Cyclopentylphenylacetic	2·6	4·36[6]
o-Toluic	0·25	3·90[2]	*p*-n-Hexylphenylacetic	29·8	4·36[6]
m-Toluic	0·31	4·27[2]	*p*-Cyclohexylphenylacetic	7·4	4·36[6]
p-Toluic	0·23	4·37[2]	*p*-Methoxyphenylacetic	<0·01	4·36[4]
2,4-Dimethylbenzoic	0·88	4·22[2]	*p*-n-Propoxyphenylacetic	0·21	4·36[6]
2,6-Dimethylbenzoic	0·10	3·35[2]	*p*-Phenoxyphenylacetic	0·82	4·36[6]
2,4,6-Trimethylbenzoic	0·35	3·45[2]	*p*-Fluorophenylacetic	0·01	4·25[4]
2,3,5,6-Tetramethylbenzoic	0·37	3·42[2]	*o*-Chlorophenylacetic	0·03	4·07[5]
o-Chlorobenzoic	0·05	2·89[3]	*m*-Chlorophenylacetic	0·11	4·14[5]
m-Chlorobenzoic	0·18	3·82[3]	*p*-Chlorophenylacetic	0·06	4·19[4]
p-Chlorobenzoic	0·17	4·03[3]	*p*-Bromophenylacetic	0·09	4·19[4]
			p-Iodophenylacetic	0·12	4·18[4]
Cinnamic	0·22	4·41[3]	*p*-Nitrophenylacetic	0·02	3·85[4]

1. Present results, calculated assuming total dimerization in the organic phase. Units $ml^{\frac{1}{2}}$ $\mu g^{-\frac{1}{2}}$.
2. Wilson, Gore & others (1967).
3. Fieser & Fieser (1956).
4. Kortum, Vogel & Andrussow (1961).
5. Handbook of Chemistry and Physics (1967).
6. Calculated values.

percentage absorptions (at pH 6·0) of the *p*-n-alkyl-phenylacetic and straight chain fatty acids (Beckett and Moffat, 1968, 1969). Since the log of the n-heptane-aqueous phase partition coefficients of the straight chain fatty acids, *p*-n-alkyl-phenylacetic acids, amphetamines and fenfluramines and their buccal absorption values are each

related to alkyl chain length, the buccal absorption values must also be directly related to the log of the partition coefficients (Fig. 2A, B). Although a good fit to straight lines is obtained, it is obvious that the results cannot be extrapolated for higher or lower members of the homologous series of amines or acids since more than 100 or less than 0% absorption is unattainable.

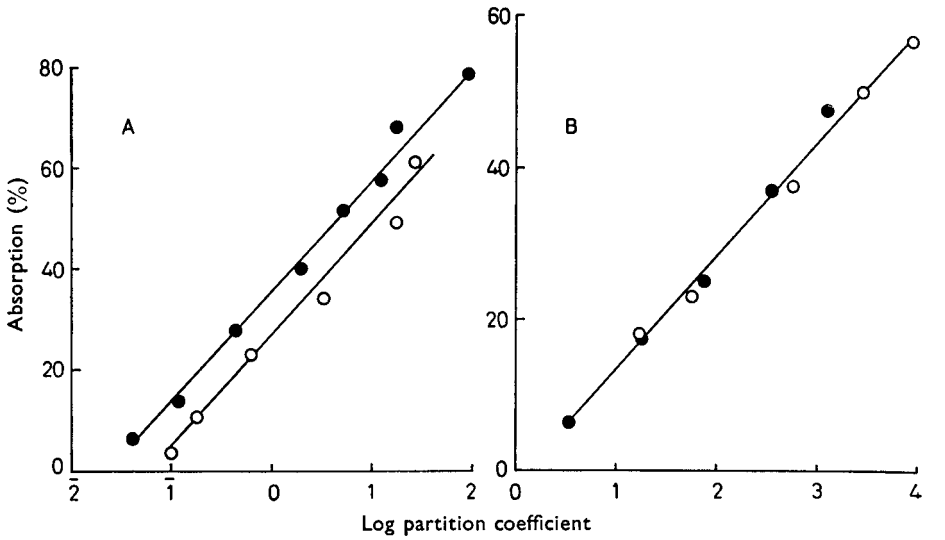

FIG. 2. A. The relation of the buccal absorption at pH 6·0 of two series of acids to their n-heptane-0·1N HCl partition coefficients. ○, *p*-n-alkylphenylacetic acids; ●, straight chain fatty acids. B. The relation of the buccal absorption of two series of *N*-n-alkyl amines (when 1% unionized) to their n-heptane-0·1N NaOH partition coefficients. ○, fenfluramines; ●, amphetamines.

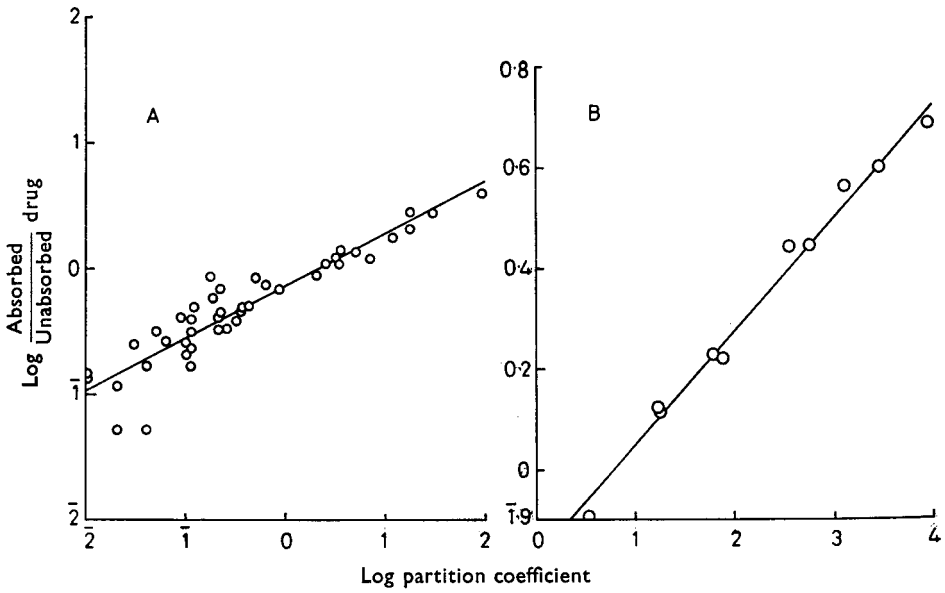

FIG. 3. A. The relation of the buccal absorption of carboxylic acids (when 10% unionized) to their n-heptane-0·1N HCl partition coefficients (correlation coefficient 0·93). B. The relation of the buccal absorption of *N*-n-alkyl substituted amines (when 10% unionized) to their n-heptane-0·1N NaOH partition coefficient (correlation coefficient 0·98).

When buccal absorptions, represented by the log of the proportion of compound absorbed, is plotted against the log of the partition coefficient a good positive correlation is obtained for the acids at 1 and 10% (correlation coefficients of 0·91 and 0·93 respectively), e.g. Fig. 3A. The lines of best fit were:

log (absorbed acid/unabsorbed acid) = 0·455 log K-0·77 (1%)

log (absorbed acid/unabsorbed acid) = 0·416 log K-0·14 (10%)

Better correlations were obtained with the amines (correlation coefficients, 0·98 and 0·98) than with the acids because only two homologous series were used, e.g. Fig. 3B. The lines of best fit were:

log (absorbed amine/unabsorbed amine) = 0·33 log K-1·12 (1%)

log (absorbed amine/unabsorbed amine) = 0·225 log K-0·174 (10%)

This indicates that the ability of any of the studied unionized amine or acid molecules to pass into the membrane is governed by their lipid solubility.

Thus n-heptane has similar partition properties to the lipid of the buccal mucosal membrane in the absorption of the acids and amines used. This is in agreement with Bickel & Weder (1969) who found that n-hexane displayed partition properties closer to the buccal mucosa, with imipramine and its metabolites, than did other less lipophilic solvents—ether, dichloroethane and chloroform. The n-heptane-aqueous system (or n-hexane-aqueous system) partition coefficient may therefore be taken to give a good indication of the degree of *in vivo* absorption of the unionized form of an acid or amine.

The pH-partition hypothesis is thus applicable to the passage of acids and bases into the buccal mucosa since when their unionized forms have the same partition coefficient, they penetrate the membrane with equal ease. For example, the unionized forms of amphetamine and *p*-n-propylphenylacetic acid have approximately the same n-heptane-aqueous phase partition coefficients (i.e. the same amount enters the n-heptane phase although the acid dimerizes in the organic solvent) and when they are 1% unionized they are absorbed by the mucosa to the extent of 6 and 15·5% respectively (a non-significant difference); methylamphetamine and *p*-cyclopentylphenylacetic acid have approximately the same K values and have similar buccal absorptions (56% and 52·5% respectively) when 10% is in the non-ionized form.

The amount of acid or amine in the unionized form is calculated from the pH of the buffer solution and the pK_a of the compound. For example, an acid pK_a 5 and an amine pK_a 9 would be 1% unionized in a buffer solution of pH 7. If they had the same partition coefficient they would be expected to be absorbed to the same extent by the buccal mucosa. However, if the pH at the site of absorption was not the same as the pH of the buffer solution, different absorptions would be observed. For instance, using the above examples, if the pH at the site of absorption was 6 whilst that of the bulk solution was 7 the acid would be 10% unionized whereas the amine would only be 0·1% unionized at the mucosa surface. Thus, since the experimental evidence quoted above indicates that acids and bases with similar partition coefficients are absorbed to the same extent when equally unionized over the pH range 9–4, the pH at the site of absorption must be the same as the measured pH of the buffer solution.

It has been shown (Beckett & Moffat, 1968) that the absorptions of 2,4,6-trimethylbenzoic and 2,3,5,6-tetramethylbenzoic acids are anomalously low when compared with benzoic acid itself. The explanation advanced was that the buffer solution pH

was not that at the buffer-buccal membrane interface where absorption took place. This is no longer tenable, and the phenomenon is due to these acids being less lipid soluble, relative to benzoic acid, than would be expected from the presence of three or four methyl groups in the aromatic ring; experimental results in confirmation are presented in Table 4.

For many organic solvents there is a direct relation between the logs of partition coefficients (organic solvent-water) of compounds in an homologous series in one solvent-water system to the logs of the partition coefficients in another solvent-water system (Collander, 1947). The partition properties of a wide variety of organic solvents would then be expected to simulate those of the buccal mucosa for members of homologous series. However, the relative magnitudes of partition coefficients for compounds of widely differing structures change when different solvents are used, especially when solvent-solute interaction takes place (Wilkinson, 1966). The use of partition coefficients in predicting absorptions of drugs may therefore give erroneous results if an unsuitable solvent is used as the lipid substitute. Even when partition experiments are made using lipid extracted from animal buccal mucosa, the K values are not necessarily valid for the living tissue (Brändström, 1964). This is because the lipid layers bind to protein and this modifies their solvent properties. The present study suggests that the buccal membrane may for all practical purposes be considered as an homogeneous lipid phase with solvent properties similar to those of n-heptane and that buccal absorption of many carboxylic acids and amines may be predicted from their pK_a values and n-heptane-aqueous phase partition coefficients.

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REFERENCES

BECKETT, A. H. & MOFFAT, A. C. (1968). *J. Pharm. Pharmac.*, **20**, *Suppl.*, 239S–247S.

BECKETT, A. H. & MOFFAT, A. C. (1969). *Ibid.*, **21**, *Suppl.*, 139S–143S.

BICKEL, M. H. & WEDER, H. J. (1969). *Ibid.*, **21**, 160–168.

BRÄNDSTRÖM, A. (1964). *Acta pharm. suecica*, **1**, 159.

BROOKES, L. G. (1968). Ph.D. Thesis, University of London.

COLLANDER, R. (1947). *Acta physiol. scand.*, **13**, 363–381.

FIESER, L. F. & FIESER, M. (1956). *Organic Chemistry*, 3rd edn, p. 163, New York: Reinhold.

Handbook of Chemistry and Physics (1967), 48th edn, p. D-90-1, Cleveland: Chemical Rubber Publishing Co.

HANSCH, C., MALONEY, P. P., FUJITA, T. & MUIR, R. M. (1962). *Nature, Lond.*, **194**, 178–180.

KNOEFEL, P. K., HUANG, K. C. & JARBOE, C. H. (1961). *J. Pharmac. exp. Ther.*, **134**, 266–272.

KNOEFEL, P. K., HUANG, K. C. & JARBOE, C. H. (1962). *Am. J. Physiol.*, **203**, 6–10.

KORTUM, G., VOGEL, W. & ANDRUSSOW, K. (1961). *Dissociation constants of organic acids in aqueous solution.* London: Butterworths.

MAYER, S., MAICHEL, R. P. & BRODIE, B. B. (1959). *J. Pharmac. exp. Ther.*, **127**, 205–211.

Merck Index (1960), 7th Edition. Rahway: Merck and Co., Inc.

REESE, D. R., IRWIN, G. M., DITTERT, L. W., CHANG, C. W. & SWINTOSKY, J. V. (1964). *J. pharm. Sci.*, **53**, 591–597.

SCHANKER, L. S. (1959). *J. Pharmac. exp. Ther.*, **126**, 283–290.

WALTON, R. P. (1935). *Proc. Soc. exp. Biol. Med.*, **32**, 1488–92.

WEINER, I. M. & MUDGE, G. H. (1964). *Am. J. Med.*, **36**, 743–762.

WILKINSON, G. R. (1966). Ph.D. Thesis, University of London.

WILSON, J. M., GORE, N. E., SAWBRIDGE, J. E. & CARDENAS-CRUZ, F. (1967). *J. chem. Soc.*, (B), 852–859.