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The influence of substitution in phenylacetic acids on their performance in the buccal absorption test

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In the buccal test there is a linear relation between percentage absorption and alkyl chain length for a series of *p*-n-alkyl phenylacetic acids. Branching and cyclizing the alkyl chain reduces the percentage absorption. With the *p*-halogeno-acids, absorption increases as the atomic weight of the halogen atom increases; a chlorine atom is approximately equivalent to a methyl group in its effect on absorption. An oxygen atom between an alkyl group and ring is equivalent to reducing the chain length by one methylene group. The absorptions of α-methyl-substituted acids are greater than those of the unsubstituted acids.

The buccal absorption test of Beckett & Triggs (1967) has been used to explore the relative importance, in buccal absorption, of pK_a and alkyl chain length in a closely related series of acids (Beckett & Moffat, 1968). The test is now used to demonstrate the relative effect of various substituents on the membrane penetrating power of phenylacetic acids.

EXPERIMENTAL

Buffer solutions in the range pH 3·0 to 9·1 (37°) were prepared using McIlvaine citric acid-phosphate buffer for pH values between 3·0 and 8·0 (Documenta Geigy, 1962a) and borax (0·05M) for pH 9·1. Sörensen phosphate buffer and borax buffer were used for propylphenylacetic acid since citric acid interfered with the analysis. All pH values were measured at room temperature with a Pye Dynacap pH meter.

The general method of Beckett & Moffat (1968) was used: gas-liquid chromatographic conditions and the internal standards used for analyses are summarized in Table 1.

RESULTS AND DISCUSSION

Two groups of acids were used, viz. (*a*) *p*-substituted phenylacetic acids which differ among themselves in their lipid solubility but not in their pK_a values and (*b*) positional isomers of some mono-substituted phenylacetic acids and some *p*-alkyl-α-methylphenylacetic acids, differing from acids of group (*a*) in both lipid solubilities and pK_a values.

The percentage buccal absorptions at pH 6·0 of the various *p*-substituted acids were found to be: hydrogen 1, nitro 1, fluoro 1·5, methoxy 3, methyl 7, chloro 7, bromo 8, n-propoxy 10, ethyl 10, iodo 10, n-propyl 25, t-butyl 25, n-butyl 34, t-pentyl 30, cyclopentyl 30, n-pentyl 49, cyclohexyl 44, n-hexyl 61.

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Table 1. *Gas-liquid chromatography conditions for the analysis of some phenylacetic acids*

Methylester of acid	Retention time (min)	Operating temp (° C)	Methylester of internal standard	Retention time (min)
Phenylacetic	6·1	100	*p*-Chlorobenzoic	9·6
o-Tolylacetic	4·7	115	Cinnamic	8·7
m-Tolylacetic	4·6	115	*p*-Toluic	3·9
p-Tolylacetic	4·6	115	*p*-Toluic	3·9
p-Ethylphenylacetic	5·7	130	*p*-Chlorobenzoic	3·1
p-n-Propylphenylacetic	15·6	125	*p*-t-Butylphenylacetic	20·3
p-n-Butylphenylacetic	4·1	160	*p*-t-Butylphenylacetic	3·2
p-t-Butylphenylacetic	20·3	125	*p*-Chlorobenzoic	3·9
p-n-Pentylphenylacetic	6·4	160	*p*-t-Butylphenylacetic	3·2
p-t-Pentylphenylacetic	48·8	125	*p*-t-Butylphenylacetic	20·3
p-Cyclopentylphenylacetic	7·9	165	*p*-t-Butylphenylacetic	3·0
p-n-Hexylphenylacetic	10·2	160	*p*-t-Butylphenylacetic	3·2
p-Cyclohexylphenylacetic	12·4	165	*p*-t-Butylphenylacetic	3·0
p-Methoxyphenylacetic	12·7	125	*p*-t-Butylphenylacetic	20·3
p-n-Propoxyphenylacetic	19·0	130	*p*-Chlorobenzoic	3·1
p-Fluorophenylacetic	2·6	125	*p*-Chlorobenzoic	3·9
o-Chlorophenylacetic	6·9	115	Cinnamic	8·7
m-Chlorophenylacetic	6·2	125	*p*-Chlorobenzoic	3·9
p-Chlorophenylacetic	7·0	125	*p*-Chlorobenzoic	3·9
p-Bromophenylacetic	11·4	125	*p*-Chlorobenzoic	3·9
p-Iodophenylacetic	20·4	125	*p*-Chlorobenzoic	3·9
p-Nitrophenylacetic	4·3	165	*p*-t-Butylphenylacetic	3·0
p-Ethyl-α-methylphenylacetic	8·0	128	*p*-Chlorobenzoic	3·4
p-n-Propyl-α-methylphenylacetic	13·6	128	*p*-Chlorobenzoic	3·4
p-iso-Butyl-α-methylphenylacetic	17·8	128	*p*-Chlorobenzoic	3·4
p-t-Pentyl-α-methylphenylacetic	27·4	128	*p*-Chlorobenzoic	3·4

Typical of the first group are the *p*-n-alkyl phenylacetic acids whose absorption increases, over the pH range 3 to 9, as the chain length increases (Fig. 1A) even though their pK_a values are approximately the same (4·31–4·37; Kortum, Vogel & Andrussow, 1961). At a single pH, e.g. 6·0 which is the mean pH of saliva (Documenta Geigy,

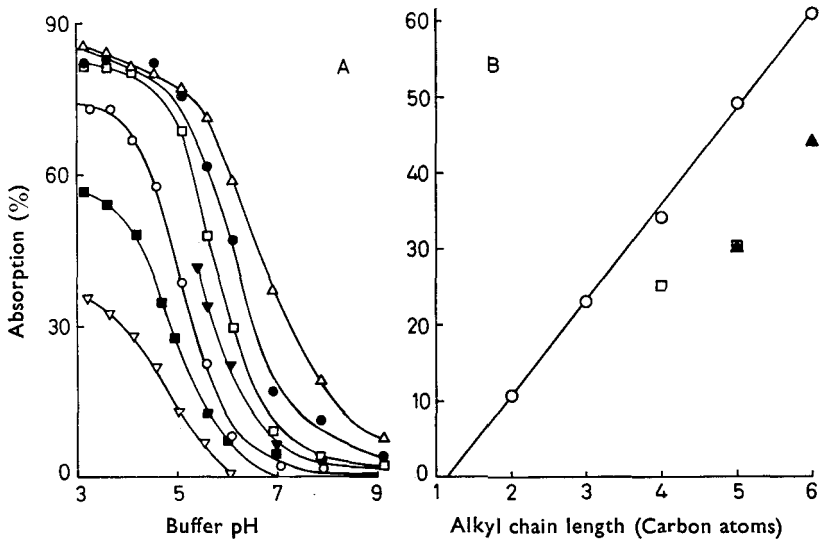

FIG. 1. A. The buccal absorption of *p*-n-alkyl phenylacetic acids (Subject I). △, hexyl; ●, pentyl; □, butyl; ▼, propyl; ○, ethyl; ■, methyl; ▽, H. B. The effect of chain length in *p*-alkyl phenylacetic acids on their buccal absorption at pH 6·0. ○, normal, □, tertiary; ▲, cyclic.

1962b), a linear relation obtains between buccal absorption and alkyl chain length of the *p*-n-alkyl phenylacetic acids (Fig. 1B). This indicates that buccal absorption is an additive property of a drug molecule. Each additional methylene group in a chain causes an increment of 12·5% in the buccal absorption. The increase resulting from the addition of a methylene group agrees with previously reported data using straight-chain fatty acids (Beckett & Moffat, 1968); the increased lipid-water partition coefficients of their unionized forms explain the results. For example, the n-heptane-0·1N hydrochloric acid partition coefficients of phenylacetic and *p*-n-hexylphenylacetic acids at 25°, assuming complete dimerization in the n-heptane phase, are 0·01 and 29·8 $ml^{1/2} \mu g^{-1/2}$ respectively (Beckett & Moffat, unpublished observations).

t-Pentyl- and cyclopentyl-phenylacetic acids are absorbed to a similar extent, but much less than the n-pentyl compound (Fig. 2A). Comparison of these results with those obtained using t-butyl-, n-butyl-, cyclohexyl-, and n-hexyl-phenylacetic acids (above and Fig. 1B) indicates that the tertiary branching or the cyclizing of an alkyl chain with the same number of carbon atoms produces a fall in absorption to the level of the next lower straight-chain homologue.

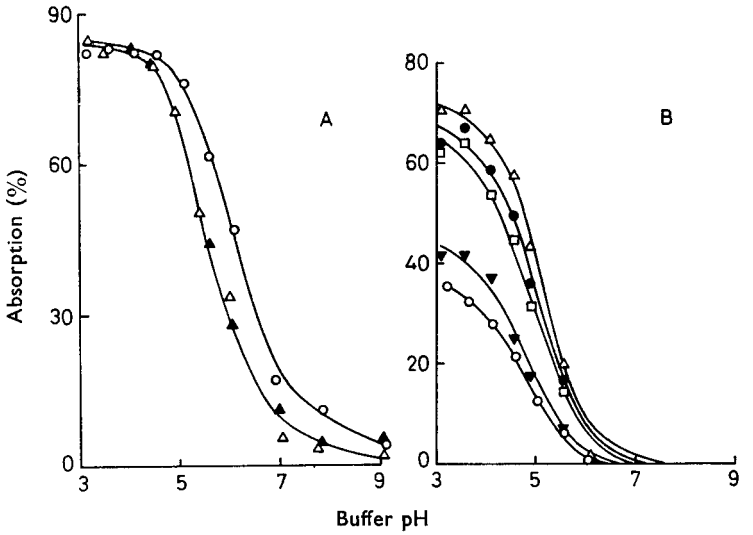

FIG. 2. A. The buccal absorption of *p*-alkyl phenylacetic acids (Subject I). ○, n-pentyl; ▲, cyclopentyl; △, t-pentyl. B. The buccal absorption of phenylacetic acid and *p*-halogen phenylacetic acids (Subject I). △, iodo; ●, bromo; □, chloro; ▼, fluoro; ,○ H.

That compounds with straight alkyl chains are absorbed to a greater extent than branched chain compounds with the same number of carbon atoms is to be expected since, in acids with chains larger than C_3, the paraffinic side-chain becomes a dominant feature (Albert, 1965); it cannot be accommodated in any interstices, it cannot force the water molecules apart and thus be solubilized, hence it tends to be "squeezed" out of the water into the mucosa, dragging the whole molecule with it. The effect is lessened by reducing the length of the chain, by branching, or forming a ring.

The percentage of *p*-halogenated phenylacetic acids absorbed increases as the atomic weight of the halogen increases (Fig. 2B). When compared with the *p*-alkyl substituted phenylacetic acids (Fig. 1A), chlorine increases buccal absorption to

approximately the same extent as a methyl group, whilst iodine is equivalent to an ethyl group. Fluoro- and bromo-groups are about half as effective in increasing absorption as the chloro- and iodo-groups respectively. This is in agreement with Brookes (1968) who found the buccal absorption of *p*-chlorophentermine to be much greater than that of phentermine.

p-Methoxyphenylacetic acid shows approximately the same percentage absorption as phenylacetic acid, and *p*-n-propoxyphenylacetic acid shows approximately the same absorption as *p*-ethylphenylacetic acid (Fig. 3A). The acids in each pair also have similar pK_a values and lipid-water partition coefficients (Beckett and Moffat, unpublished observations). Thus, insertion of an oxygen atom between the alkyl chain and the phenyl ring produces an effect approximately equal to reducing the chain length by one methylene group.

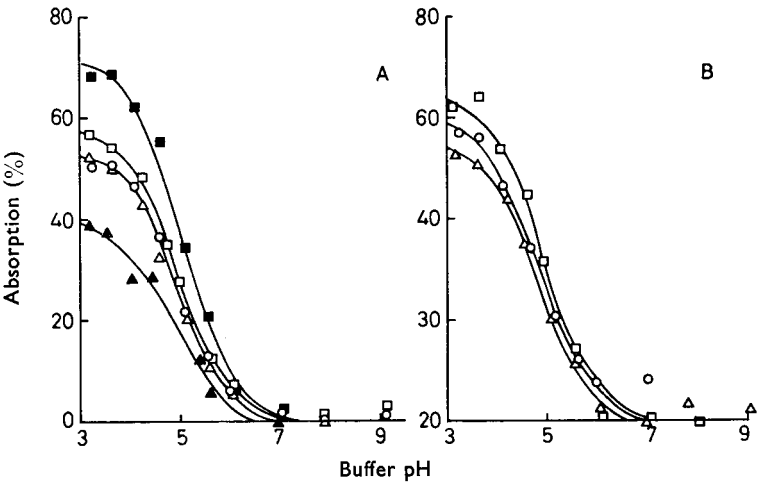

FIG. 3. A. The buccal absorption of some *p*-alkoxy and *p*-alkyl phenylacetic acids (Subject I). ■, propoxy; ▲, methoxy; □, *p*-methyl (pK_a 4·37); ○, *m*-methyl; △, *o*-methyl (pK_a 4·35). B. The buccal absorption of the monochlorophenylacetic acids (Subject I). □, *para* (pK_a 4·19); ○, *meta* (pK_a 4·14); △, *ortho* (pK_a 4·07).

With the three tolylacetic and three monochlorophenylacetic acids (Fig. 3A, B) the shifts in the curves indicate that the differences in buccal absorption are primarily due to their pK_a differences.

The *p*-alkyl-α-methylphenylacetic acids are absorbed more than their *p*-alkyl-phenylacetic acid homologues (cf. Figs 1A and 4); the relative percentage absorptions for the ethyl-, n-propyl- and t-pentyl-α-methyl acids and their homologues being 33·5/10, 38/23 and 40/30 respectively at pH 6·0. The absorption of *p*-isobutyl-α-methylphenylacetic acid, for which no comparable non-α-methyl compound was available, was 44% at pH 6·0. Although the pK_a values of phenylacetic acid and α-methyl-phenylacetic acid differ (4·28 and 4·64 respectively: Handbook of Chemistry and Physics, 1967), and this would account for some of the absorption differences between the respective homologues (Beckett & Moffat, 1968), it is possible that the absorption differences are also due to differences in lipid solubility of the unionized forms.

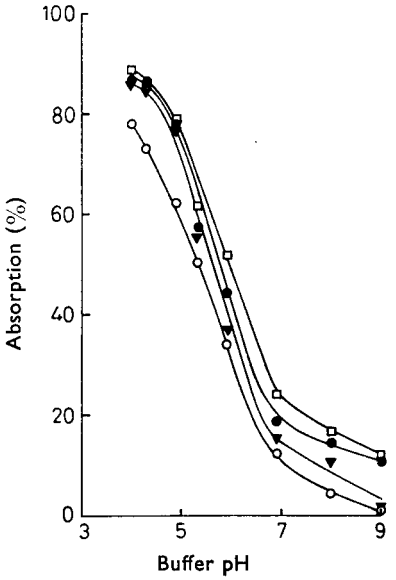

FIG. 4. The buccal absorption of *p*-alkyl-α-methylphenylacetic acids (Subject I). ○, ethyl; ▼, n-propyl; □, isobutyl; ●, t-pentyl.

The absorptions of phenylacetic acids therefore alter by changing either the subst tuent or its position in the molecule. The addition of alkyl chains to the acid gives the greatest increase in absorption, although straight alkyl chains on aromatic rings of drug molecules are susceptible to β-oxidation by enzymes, whereas branched chains are relatively much more stable. Thus, to make a drug more lipid soluble, so that it is absorbed quickly and reaches its site of action in the shortest time, the addition of a multi-branched or cyclic alkyl chain may give the best result. As an example, *p*-isobutyl-α-methylphenylacetic acid is used as an anti-inflammatory agent whilst phenylacetic acid is inactive.

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