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STRUCTURE-ACTIVITY RELATIONS—III. BRONCHODILATOR ACTIVITY OF SUBSTITUTED 6-THIOXANTHINES

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Abstract—A correlation of the bronchodilator activity of a series of substituted 6-thioxanthines has been made with partition parameters and/or the steric "bulk" of the 1- and 3-substituents. The 3-substituted series are correlated by a Hansch relation involving partition factors alone. Steric factors appear to be important for substitution at the 1-position. In general, an increase in activity is observed on introduction of "bulky" substituents at both these positions. The significance of these results is discussed.

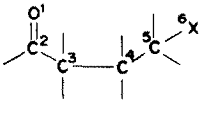
A QUANTITATIVE analysis of structure–biological activity relations was introduced by Hansch.[1,2] This treatment employed substituent constants to describe the polar, partition and other related properties controlling the activity under study. A number of factors have been shown to be important. The original Hansch equations (1) and (2), or simplified versions can be used to correlate the molar concentration, C_x, of a

$$\log (1/C_x) = -a\pi^2 + b\pi + \rho\sigma + C \tag{1}$$

$$\log (1/C_x) = b\pi + \rho\sigma + C \tag{2}$$

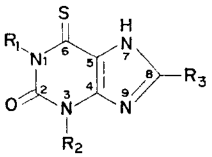
substituted compound of a series, all causing an equivalent biological response, to a hydrophobic bonding or partition constant, π, and a Hammett constant, σ.[1,2] Other structural parameters can be used instead of π or σ, or in company with one or both of them. Recently, steric effects have received attention in a number of studies.[3–6] While some success has been achieved, it is unlikely that a generalized approach to steric effects can be made because of their diverse nature and mode of their operation in control of activity. The steric parameter, E_s, obtained from the Taft treatment[7] will be applicable only to systems where the latter parameter correctly simulates the type of steric effect involved. Likewise parameters based on Van der Waal's radii[6,8] will be useful if the "bulk" property they describe is implicated. Newman[9] has introduced his concept of "six-number" to evaluate the steric effect caused by "bulk" interactions which inhibit approach to a reaction centre arising from "coiled" chains. The number of atoms at the six position can be evaluated as, for example, in I below.

This parameter has been successfully used in quantitative correlations of chemical reactivity.[10]



(I)

A series of substituted 6-thioxanthines (II) have been shown to have potent bronchodilator activity.[11]



(II)

A comprehensive series of compounds, having a wide range of alkyl and related substituents at the 1- and 3-positions, has been examined. In this study we describe an analysis of the structure–activity relations in terms of structural parameters.

RESULTS AND DISCUSSION

In Table 1 are shown the values of log (1/*C*), relative to that of 1,3-dimethyl-6-thioxanthine as 0·0, for the bronchodilator activity of the series of twenty-five 6-thioxanthines under study. The compounds were tested as their choline (Table 1; compounds 1–8, 10–16, 18, 21 and 22) or sodium (Table 1; compounds 9, 17, 19, 20 and 23) salts. This difference should not cause any major discrepancy[11,12] and has been ignored in the analysis. The method used for estimating bronchodilator activity on isolated guinea-pig tracheal ring preparation has been fully described.[11–13] The number

TABLE 1. BRONCHODILATOR ACTIVITY AND SUBSTITUENT CONSTANTS FOR THE SUBSTITUTED 6-THIOTHEOPHYLLINES (II)

				Bronchodilator activity		"Six-number"			
	R_1	R_2	R_3	Log (1/*C*)	π	$_1N_C$	$_1N_H$	$_2N_C$	$_2N_H$
1	Me	Me	H	0·00	1·00	0	0	0	0
2	Me	CH_2Me	H	0·60	1·50	0	0	0	3
3	Me	$(CH_2)_2Me$	H	$0{\cdot}94_5$	2·00	0	0	1	2
4	Me	$CH_2CH{=}CH_2$	H	$0{\cdot}41_5$	1·70	0	0	1	1
5	Me	$(CH_2)_3Me$	H	$1{\cdot}02_5$	2·50	0	0	1	2
6	Me	CH_2CHMe_2	H	1·16	2·30	0	0	2	1
7	Me	$CH_2CMe{=}CH_2$	H	$0{\cdot}85_5$	2·00	0	0	2	0
8	Me	$(CH_2)_4Me$	H	1·07	3·00	0	0	1	2
9	Me	$(CH_2)_3OMe$	H	0·60	1·53	0	0	1	2
10	Me	$(CH_2)_2CHMe_2$	H	$1{\cdot}03_5$	2·80	0	0	1	2
11	Me	$(CH_2)_5Me$	H	0·66	3·50	0	0	1	2
12	Me	CH_2Ph	H	$0{\cdot}76_5$	3·20	0	0	2	0
13	Me	$(CH_2)_2Ph$	H	$0{\cdot}64_5$	3·70	0	0	1	2
14	Me	Furfuryl	H	0·30	2·48	0	0	1	1
15	CH_2Me	Me	H	$0{\cdot}25_5$	1·50	0	3	0	0
16	CH_2Me	CH_2Me	H	$0{\cdot}85_5$	2·00	0	3	0	3
17	CH_2Me	$CH_2CH{=}CH_2$	H	$0{\cdot}55_5$	2·20	0	3	1	1
18	CH_2Me	$(CH_2)_3Me$	H	1·14	3·00	0	3	1	2
19	CH_2Me	CH_2CHMe_2	H	$1{\cdot}45_5$	2·80	0	3	2	1
20	$CH_2CH{=}CH_2$	Ph	H	0·99	3·33	1	1	0	4
21	$(CH_2)_2Me$	$(CH_2)_2Me$	H	$1{\cdot}45_5$	3·00	1	2	1	2
22	$(CH_2)_3Me$	Me	H	$-0{\cdot}05_5$	2·50	1	2	0	0
23	$(CH_2)_3Me$	$(CH_2)_3Me$	H	$1{\cdot}60_5$	4·00	1	2	1	2
24	Me	Me	Me	0·00	1·50				
25	Me	Me	CH_2Me	0·34	2·00				

of experiments by Armitage *et al.*[11–13] for each compound varied from 3 to 17 with small ranges of activity values for all the compounds except 11, 22 and 24, Table 1 (2 experiments only with a very small range) and 14, Table 1 (1 experiment). The value for the latter compound thus must be reviewed with caution.

The substituent π values, obtained from Hansch's studies,[14–17] are also shown in Table 1, assuming simple additivity between the separate "non-proximate" positions.[14–16] The Newman "six-number" values, shown in Table 1, are designated for each substituent as ${}_xN_y$ values, being the number of atoms at the six position, numbering from the carbonyl oxygen atom as 1. The subscript x as 1 or 2 refer to the substituent in the xanthine ring, R_1 or R_2 in II, and y to the nature of the atom at the six position, i.e. C and H for carbon and hydrogen, respectively. The "six-number" for the furfuryl, benzyl and phenyl groups (compounds 14, 12 and 20 in Table 1) cannot be calculated directly and were estimated by comparison of "Catalin" molecular models of these substituents with those of alkyl groups. The steric substituent constants, E_s,[7] are available for most of the substituents under consideration and a value for the allyl group can be calculated as −0·14 from literature data[9] (eleven of the fifteen substituents included). The polar substituent constants, σ^I,[7,18] are available for all the fifteen different substituents under consideration. However, with the exception of two examples, the range of these values is very small, i.e. σ^I between 0·10 and −0·05. Correlations employing σ^I values were attempted and indicated no significant contribution from this parameter. In Table 2, the relation between the various parameters under consideration are shown. The only significant relation is that between the E_s and "six-number" parameters, which measure a closely related steric "bulk" property of the substituents. The latter steric parameters were therefore not used together in a correlation. Although similar correlations were obtained using either steric parameter, the "six-number" parameters were more successful.

TABLE 2. RELATIONS BETWEEN THE VARIOUS STRUCTURAL PARAMETERS USED IN THESE STUDIES*

		n	*s*	*r*
1	$\pi = 0{\cdot}395\ {}_{1,2}N_{C,H}\ (1{\cdot}88) + 0{\cdot}861$	15	0·716	0·463
2	$E_s = -0{\cdot}137\ {}_{1,2}N_{C,H}\ (1{\cdot}87) + 0{\cdot}006$	11	0·217	0·529
3	$\pi = 1{\cdot}637\ E_s\ (1{\cdot}55) + 1{\cdot}410$	11	0·807	0·460
4	$\pi = 0{\cdot}790\ N_C\ (2{\cdot}28) + 0{\cdot}395\ N_H\ (1{\cdot}95) + 0{\cdot}466$	15	0·691	0·570
5	$E_s = -0{\cdot}347\ N_C\ (4{\cdot}62) - 0{\cdot}075\ N_H\ (1{\cdot}47) + 0{\cdot}119$	11	0·142	0·853

* *n* = Number of compounds; *r* = correlation coefficient; *s* = standard deviation and the quantities in parenthesis are the Student's *t*-test (lit. G. W. Snedecor, *Statistical Methods*, 5th ed., Iowa State University Press, Ames, Iowa, 1956) for the significance of the regression variable.

The correlations obtained are shown in Table 3. Firstly, for *all* the compounds there is no discernible dependence on the partition constant, π, whether used alone or in combination with the steric "bulk" parameters, e.g. equations 1, 2 and 10, Table 3. However, a series of compounds comprising all those with R_1 and R_3 equal to Me and H, respectively, excepting compound 14, Table 1, do give an excellent correlation with partition factors alone, i.e. equation 11, Table 3. The activity of compound 14, Table 1,

TABLE 3. CORRELATIONS OF BRONCHODILATOR ACTIVITY FOR THE SUBSTITUTED 6-THIOTHEOPHYLLINES*

		n	s	r	Compounds (Table 1)
1	−0·109π^2 (0·92) + 0·899π (1·50) − 0·735	25	0·373	0·624	All
2	−0·103π^2 (0·85) + 0·829π (1·35) − 0·574	23	0·375	0·582	1–23
3	−0·407π^2 (7·16) + 2·131π (7·72) − 1·751	13	0·124	0·934	1–13
4†	−0·455π^2 (6·52) + 2·307π (7·44) − 1·776	24	0·226	0·882	All except 14
5†	−0·451π^2 (6·88) + 2·360π (7·91) + 0·088 ${}_{1}N_{C,H}$ (2·85) − 2·024	22	0·195	0·910	1–13, 15–23
6†	−0·464π^2 (6·98) + 2·397π (7·90) − 0·912 E_s (1) (2·68) − 2·000	22	0·199	0·907	As above
7	0·171 ${}_{1,2}N_{C,H}$ (4·07) + 0·186	23	0·336	0·664	1–23
8	0·463 ${}_{1,2}N_{C}$ (5·39) + 0·142 ${}_{1,2}N_{H}$ (4·14) − 0·058	23	0·266	0·817	1–23
9	0·322 ${}_{2}N_{C,H}$ (6·08) + 0·095 ${}_{1}N_{C,H}$ (2·42) − 0·096	23	0·266	0·816	1–23
10	0·440 ${}_{1,2}N_{C}$ (3·94) + 0·136 ${}_{1,2}N_{H}$ (3·52) + 0·032π (0·33) − 0·101	23	0·272	0·818	1–23
11	0·536 ${}_{2}N_{C}$ (7·22) + 0·278 ${}_{2}N_{H}$ (6·29) + 0·123 ${}_{1}N_{C,H}$ (3·80)	23	0·213	0·893	1–23
12	0·547 ${}_{2}N_{C}$ (7·37) + 0·269 ${}_{2}N_{H}$ (6·05) + 0·264 ${}_{1}N_{C}$ (2·08) + 0·102 ${}_{1}N_{H}$ (2·77) − 0·255	23	0·211	0·901	1–23
13	−1·319 E_s (2) (4·66) − 0·931 E_s (1) (1·70) + 0·328	19	0·317	0·768	1–6, 8, 10–13, 15–19, 21–23
14	−0·834 E_s (2) (3·40) + 1·13 E_s (1) (1·66) + 7·97 E_s (1).E_s (2) (3·78) + 0·483	19	0·234	0·889	As above

* See Table 2.

† π Values for R_2 (with R_1 = Me).

is based on only one measurement unlike the other compounds in this study[11–13] (see above). The divergence between the activity of the latter compound and its calculated value based on the correlation of the other compounds caused us to reject it in further regression involving π values. The value of π_0 for maximum bronchodilator activity in this selected series is 2·6.

All terms in this relation have the Student's t-test indicating significance at greater than the 99·9 per cent level and the mean deviation between calculated and observed values of log $(1/C)$ is $\pm$ 0·08. This prompted us to consider just the π value for the substituent R_2 for *all* the compounds in Table 1. A good correlation results, equation 4, Table 3, and clearly indicates an apparent unimportance of partition factors resulting from substitution at positions other than R_2. Combination of the latter partition factor treatment with steric "bulk" parameters referring to R_1 gives two very successful relations, equations 5 and 6, Table 3. The slightly more successful first of these two relations has a π_0 value (referring only to R_2) close to that found for equation 4, Table 3, and the partition and steric "bulk" terms have the Student's t-test indicating significance at greater than the 99·9 and 98·5 per cent levels, respectively. The mean deviation between calculated and observed values of log $(1/C)$ is $\pm$ 0·13, in a range of 1·66. While we have employed simple additive contributions to evaluate π for substitution at both R_1 and R_2, we appreciate that both intramolecular interactions between R_1 and R_2 could perturb additivity and shielding of adjacent polar functional groups could increase the effective hydrophobicity.[19] Studies of the substituent effects on the partition characteristics of 6-thioxanthines are being made to evaluate the importance of such factors.*

Secondly, the use of the "six-number" parameters provides interesting relations. In equation 7, Table 3, the activities are related to the latter parameters without distinction as between the position of substitution or type of atom present at the six position, i.e. ${}_{1,2}N_{\mathrm{C,H}}$. The latter correlation is distinctly improved by separating the contributions either into those from carbon and hydrogen atoms at the six position, i.e. ${}_{1,2}N_{\mathrm{C}}$ and ${}_{1,2}N_{\mathrm{H}}$, as in equation 8, Table 3, or into those from the substituents R_1 and R_2, i.e. ${}_{1}N_{\mathrm{C,H}}$ and ${}_{2}N_{\mathrm{C,H}}$, as in equation 9, Table 3. A significant further improvement arises when the "six-number" parameters are separated into contributions from carbon and hydrogen atoms at the six position for R_2 and a combined contribution for R_1, i.e. ${}_{2}N_{\mathrm{C}}$, ${}_{2}N_{\mathrm{H}}$, and ${}_{1}N_{\mathrm{C,H}}$, as in equation 11, Table 3. This is very successful in accounting for the effects of substitution on activity. All terms in the latter equation have the Student's t-test indicating significance at greater than the 99·9 per cent level and the mean deviation between calculated and observed values of log $(1/C)$ is $\pm$0·16, in a range of 1·66 units. Further separation of the contribution from the carbon and hydrogen atoms at the six position results in a correlation (equation 12, Table 3) which has a slightly improved correlation coefficient and standard deviation. However, the confidence levels for the terms arising from ${}_{1}N_{\mathrm{C}}$ and ${}_{1}N_{\mathrm{H}}$ are significant only at the >95 and $>97·5$ per cent levels, respectively, although the relative magnitude of these terms is of interest. The reality of these observations is confirmed as the carbon atom has about 2–3 times the influence of a hydrogen atom at the six position at both R_1 and R_2, in accord with their relative space-filling capacities.[20] Correlations employing E_s values[7] (equation 13, Table 3) confirm the conclusions from the relations using the "six-number" parameters as in

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* K. Bowden, unpublished results.

equation 9, Table 3. A further correlation, equation 14, Table 3, employs the product E_s (1) × E_s (2) term. This successful correlation indicates the importance of the combination of "bulk" at both R_1 and R_2.

The conclusions arising from this analysis appear to be that the bronchodilator activity of these compounds can be accounted for in terms of partition and steric "bulk" factors. Partition factors appear to be only important for substitution at R_2, while increasing steric "bulk" substitution at R_1 *increase* activity. In general, there is an increasing activity with increasing "bulk" substitution. The partition contribution from the substituent R_2 alone could arise from this substituent only being "removed" from the aqueous environment; while, the steric factor for the substituent R_1 could possibly result from shielding of a xanthine carbonyl or thiocarbonyl group.

There appears to be little evidence relating to the mode of action as bronchodilators of 6-thioxanthines or xanthine derivatives, in general. Theophylline (1,3-dimethylxanthine), as its ethylenediamine salt (aminophylline), has been found to partially mediate its bronchodilator action via the adrenal glands.[21] However, the study of adrenergic antagonists towards the compound indicated a dual action, both sympathetic and direct.[21] Sutherland *et al.*[22–23] have offered a hypothesis that the bronchodilator action of theophylline and other xanthines arises from their ability to inhibit cyclic phosphodiesterase and increase the level of cyclic 3′,5′-AMP. A very recent study[25] of the *in vitro* inhibition of cyclic 3′,5′-AMP phosphodiesterase by a series of 8-substituted theophyllines has certain parallels with the present study of the bronchodilator activity of 1,3-substituted 6-thioxanthines. Partition factors do not appear to be a prime factor in controlling the activity of the 8-substituted theophyllines; while the "size" of the 8-substituent does appear to be important in increasing the inhibitory power of the derivative.[25] Studies on the inhibitory effects of these 6-thioxanthines on the action of cyclic 3′,5′-AMP phosphodiesterase are in progress.

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