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Structure activity relation for some 1,4-benzodiazepinones: correlation between rate constants for reduction by sodium borohydride and antileptazol ED50

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Significant correlation in 11 different 1,4-benzodiazepinones has been established between log k_2 (the second order rate constant for the reduction of the 'azepinones' by sodium borohydride) and their ED50 against leptazol-induced seizures in mice. The results suggest a possible involvement of the carbonyl group at the receptor site.

1,4-Benzodiazepinones ('azepinones') were first exploited by Randall (1961) in his studies on chlordiazepoxide. Comprehensive reviews have since appeared (Svenson & Gordon, 1965; Zbinden & Randall, 1967; Clifford & Franklin Smyth, 1974; Randall, Schallek & others, 1974). The purpose of this investigation was to determine whether any correlation exists between the rate of reduction of the 'azepinones' using sodium borohydride ($NaBH_4$) and any of the reported pharmacological properties such as ED50 against leptazol-induced seizures in mice, LD50's in rats and mice, and anti-maximal and anti-minimal electro-shock ED50's.

MATERIALS AND METHODS

Sodium borohydride (Fisher Scientific Company) was used without further purification. A solution of sodium borohydride (0·012 M) in isopropanol was standardized by the iodate method (Lyttle, Jensen & Struck, 1952).

Isopropanol (Fisher Scientific Company) was dried and distilled before use: those fractions distilling between 81° and 82° were used.

All the 'azepinones' used (Table 1) were kindly provided by Dr Jack B. Crosby (Hoffmann-La Roche, Inc., Houston, Texas) and Dr William Scott (Hoffmann-La Roche, Inc., Nutley, New Jersey) and were used without further purification.

Rate measurements

An accurately weighed quantity of the 'azepinone' was dissolved in isopropanol (90 ml) in a well-stoppered reaction bottle; the solution was equilibrated at 45° (2 h). Sodium borohydride solution (10 ml), at the same temperature, was then added with a rapid delivery pipette, the time of half delivery being taken as zero time. The reaction bottle was well shaken as rapidly as possible to ensure thorough mixing of the reactants; the reaction was followed by withdrawing 5 ml aliquots at various time intervals, these were quenched in potassium iodate (10 ml of 0·005 M) solution, potassium iodide (10 ml of 5%) and dilute sulphuric acid (10 ml of 2·5 M) solutions were added and the iodine liberated was titrated against standard sodium thiosulphate. The concentration of unreacted sodium borohydride in each aliquot was determined from the concentration of iodate consumed.

It is known (Brown, Wheeler & Ichikawa, 1957) that 4 mol of carbonyl group react with each mol of borohydride, the overall reaction rate being expressed by

$$\frac{dx}{dt} = k_2(a - 4x)(b - x) \quad .. \quad (1)$$

where a and b are the initial molar concentrations of 'azepinone' and sodium borohydride respectively and x the concentration of sodium borohydride which has reacted at time t. The integrated form of this equation is:

$$\log \frac{b(a - 4x)}{a(b - x)} = \frac{k_2(a - 4b)}{2{\cdot}303} t \quad .. \quad (2)$$

the second order rate constant, k_2, for the reduction of each 'azepinone' being determined from the slope of a plot of $\log[b(a - 4x)/a(b - x)]$ *vs* t (Fig. 1) since

$$k_2 = \frac{2{\cdot}303 \text{ slope}}{(a - 4b)} \quad .. \quad .. \quad (3)$$

* Correspondence.

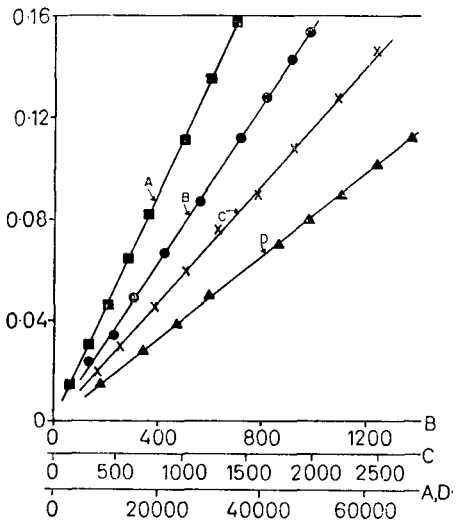


FIG. 1. Second order kinetics plot for the sodium borohydride reduction of some 1,4-benzodiazepinones. A, Fluorazepam; B, clonazepam; C, nitrazepam; D, diazepam [identification nos in Table 1: 10, 2, 6, 11 respectively]. Ordinate: Log [b(a − 4x)/a(b − x)]. Abscissa: Time (s).

Product analysis

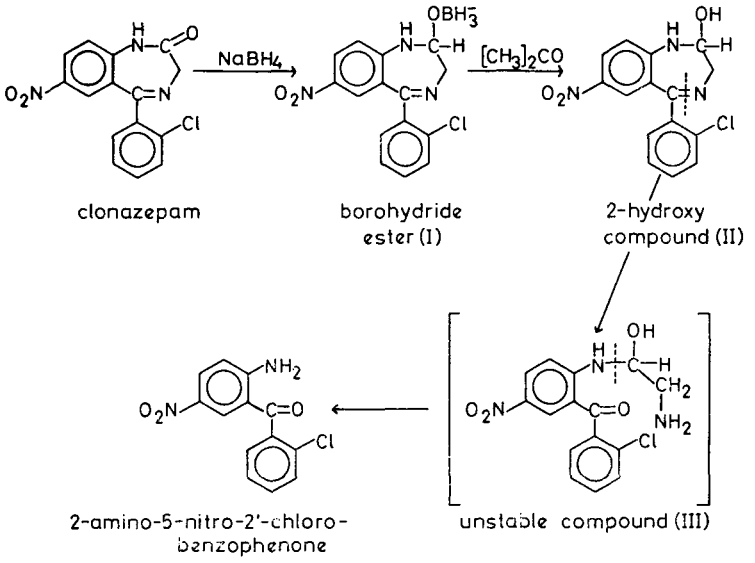
Typical product analysis is described for clonazepam. Clonazepam (0·01 M in 50 ml isopropanol) was allowed to react with sodium borohydride (0·003 M in 50 ml isopropanol) at 45° for 3 h. Excess borohydride and the borohydride complex formed were hydrolysed using 5 ml of acetone and the solvent removed under vacuum using a rotary evaporator. The products were then separated by t.l.c. using silica gel plates with ethylacetate–chloroform (90 : 10 v/v) as the running solvent. The product (98% yield) obtained from clonazepam gave only one spot (R_F 0·82) which was identified as 2-amino-5-nitro-2′-chlorobenzophenone from infrared and mass spectral data (courtesy of Drs Ian Fryer and William Scott of Hoffmann-La Roche, Inc., Nutley, New Jersey).

The reaction sequence for the formation of 2-amino-5-nitro-2′-chlorobenzophenone from clonazepam is shown in Scheme 1.

With diazepam, which reacts slowly with $NaBH_4$, the corresponding alcohol (similar to II) has also been isolated and identified. This clearly indicates that in the solvent medium (100% isopropanol) used in the kinetic experiments, the initial reaction should be the formation of the corresponding borohydride ester of the 2-hydroxybenzodiazepine (similar to I) in each of the 'azepinones' studied.

RESULTS

Table 2 gives the second order rate constants with several pharmacological parameters for the eleven different 'azepinones' studied. The antileptazol ED50 values are those of Randall & others (1974). The linearity of the plots of log [b(a − 4x)/a(b − x)] *vs* time t (Fig. 1) indicates that the reaction in each case is entirely second order. The relation between antileptazol ED50 and log k_2 (as shown in Fig. 2) is



Scheme 1

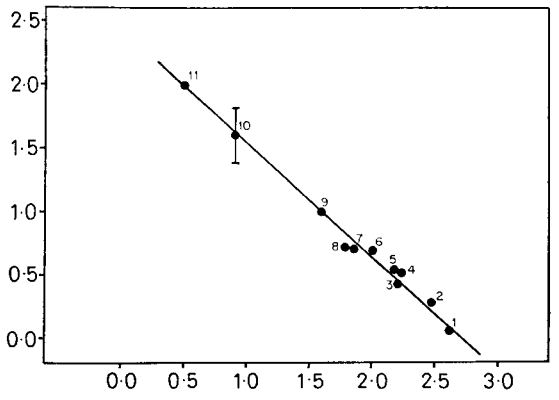


FIG. 2. Linear regression between log k_2 and antileptazol ED50. For drug numbers and structures, refer to Table 1. Ordinate: Antileptazol ED50. Abscissa: 3 + log k_2.

expressed by the least mean square regression equation

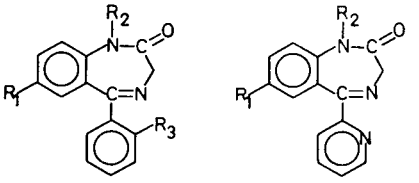
$$y = 2{\cdot}40 - 0{\cdot}879x \; (r^2 = 0{\cdot}99, n = 11) \qquad (4)$$

DISCUSSION

The structural and physico-chemical parameters which strongly influence pharmacological or biological activity are π, the lipophilicity (Hansch, Maloney & others, 1962; Fujita, Iwasa & Hansch, 1964; Leo, Hansch & Elkins, 1971; Leo, Jow & others, 1975); σ, the substituent constant (Hammett, 1970); E_S, the steric constant (Taft, 1956); and pKa (Bell & Roblin, 1942; Yamazaki, Nobuharu & others, 1970). Other factors include the molecular geometry determined from molecular orbital calculations (Germer, 1973; 1974) and Rm, determined from thin-layer chromatography (Biagi, Barbaro & others, 1974; Biagi, Barbaro & others, 1975a; Biagi, Gandolfi & others, 1975b).

Few attempts (Osterman-Golkar, Ehrenberg & Wachtmeister, 1970; Purcell, Bass & Clayton, 1973; Ehrenberg, Osterman-Golkar & Singh, 1974; Osterman-Golkar, 1976; Trieff, Venkatasubramanian & others, 1976) have been made to correlate chemical reaction rates, which are easier to determine than the above mentioned molecular parameters, with biological or pharmacological activities. Since the rate of any reaction for a particular series of compounds having the same parent structure can be correlated with the Hammett σ constants (Taft, 1956; Leffler & Grunwald, 1963; Hammett, 1970; Chapman & Shorter, 1972) as well as other molecular parameters such as pKa, E_S, etc., reaction rates can be directly related to the biological or pharmacological activities (Hansch, 1968, 1971 & 1976; Purcell, Singer & others, 1970; Stockdale & Selwyn, 1971; Hansch, Schaeffer & Kerley, 1972; Fastrez & Fersht, 1973; Hansch, Leo & others, 1973).

Table 1. *Azepinones used.*



Identifying No. for Fig. 2	Drug No.		Structure
1	Ro 5-4200	I	$R_1 = NO_2$; $R_2 = CH_3$; $R_3 = F$
2	Ro 5-4023	I	$R_1 = NO_2$; $R_2 = H$; $R_3 = Cl$
3	Ro 5-3448	I	$R_1 = Cl$; $R_2 = CH_3$; $R_3 = Cl$
4	Ro 5-3027	I	$R_1 = Cl$; $R_2 = H$; $R_3 = Cl$
5	Ro 5-3590	I	$R_1 = NO_2$; $R_2 = H$; $R_3 = CF_3$
6	Ro 5-3059	I	$R_1 = NO_2$; $R_2 = H$; $R_3 = H$
7	Ro 5-2904	I	$R_1 = CF_3$; $R_2 = H$; $R_3 = H$
8	Ro 5-3350	II	$R_1 = Br$; $R_2 = H$
9	Ro 5-4528	I	$R_1 = CN$; $R_2 = CH_3$; $R_3 = H$
10	Ro 5-6901	I	$R_1 = Cl$; $R_2 = CH_2\text{-}CH_2\text{-}N(C_2H_5)_2$; $R_3 = F$
11	Ro 5-2807	I	$R_1 = Cl$; $R_2 = CH_3$; $R_3 = H$

The mechanism of sodium borohydride reduction of ketones proceeds through an equilibrium complex between the carbonyl and borohydride (Brown & others, 1957). The observed rate constant may represent either the rate of the direct reaction of borohydride ion with the carbonyl group (equation 5)

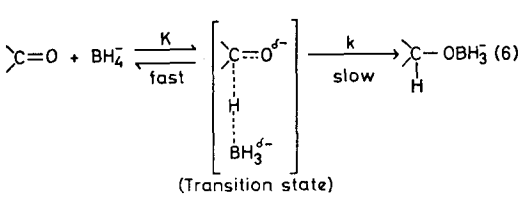
$$\text{C=O} + BH_4^- \xrightarrow{k_2} \text{C–OBH}_3^- \qquad (5)$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\quad H$$

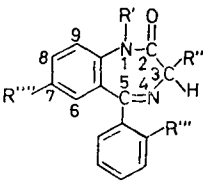
or the product of an equilibrium constant for the association of borohydride ion and the ketone, and the rate constant for the subsequent transfer of a hydride ion to the carbonyl group ($k_2 = Kk$), (equation 6). It has been observed (Warren & Parry,

Table 2. *Correlation of log k_2 for $NaBH_4$ reduction of various* 1,4-*benzodiazepinones* vs *various pharmacologica l parameters*.*

	Kinetic and pharmacological parameters											
Drug Numbers**	1	2	3	4	5	6	7	8	9	10	11	r^2†
Second order rate constant for the reduction $k_2 \times 10^3$ (litre mol^{-1} s^{-1})	419	300	160	174	152	103	72·5	63·1	39·9	8·06	3·16	
Cat muscle relaxant (mg kg^{-1}, orally)	0·02	0·05	0·1	0·1	0·05	0·1	0·1	0·2	0·5	2	0·2	0·335
Mouse LD50 (mg kg^{-1}, orally)	1380	4000	—	940	4000	2300	—	3200	—	660	970	0·249
Rat LD50 (mg kg^{-1}, orally)	485	3000	—	1550	4000	825	—	—	—	1300	710	0·112
Anti-maximal electroshock	12	21	15	0·9	12	31	7	34	15	82	22	0·293
Antileptazol ED50	0·048	0·280	0·420	0·513	0·540	0·690	0·700	0·715	0·990	1·6 ±0·22	2·0	0·989

* From Randall, Schallek & others (1974).
** For structures refer to Table 1.
† Coefficient of determination for the plot of log k_2 *vs* pharmacological parameters.





1965; Bruce, Cooksey & Morgan, 1975) that when introduced into the unsubstituted 1,4-benzodiazepinones, electron withdrawing substituents increase the rate of reduction; they also (at position 7) increase biological activity. Electron-donating substituents decrease both the rate of borohydride reduction and the biological activity (Sternbach, Randall & others, 1968).

Using a closely controlled series of drugs (Tables 1 and 2) a strong correlation has been demonstrated between the anti-leptazol ED50 values and log k_2 for the borohydride reduction of the carbonyl group in these 'azepinones'. Thus it is possible that the transition state between the 'azepinione' and the receptor is similar to the transition state occurring in the borohydride reduction of the 'azepinone'.

Of the other parameters examined, i.e., mouse LD50, rat LD50, cat muscle relaxant (orally), anti-maximal and anti-minimal electroshocks, none correlate well with the rate of borohydride reduction (Table 2).

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