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ASSOCIATION OF CNS ACTIVE DRUGS WITH 9-ETHYLADENINE

P. R. ANDREWS and A. S. BUCHANAN

Chemistry Department, University of Melbourne, Parkville, Victoria 3052, Australia

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Abstract—The association constants for the dimerization of a series of β,β-disubstituted glutarimides, and for the association of these CNS active molecules with 9-ethyladenine, have been measured in chloroform using an infrared method. All of the associations are cyclic and changes in the substituent groups which markedly effect physiological activity do not significantly influence the observed association constants. The results support a possible mode of action for the glutarimides in the respiratory chain, but do not explain their convulsant or anticonvulsant activities.

BARBITURATES are known to form strong and specific hydrogen bonds with 9-ethyladenine,[1,2] and it has been suggested that their physiological activities may be related to this interaction.[2] In particular, a planar hydrogen bonded form of the coenzyme flavinadenine dinucleotide has been proposed as a possible substrate,[3] although FAD is more commonly thought to have a folded form[4–7] in aqueous solution. However, bonding between a barbiturate and the adenine moiety would be likely to exert some influence regardless of conformation, and in the particular case of direct inhibition of the respiratory chain by barbiturates[8] such an influence on FAD, or on nicotinamide-adenine dinucleotide, could presumably be relevant. On the other hand, it seems unlikely that either coenzyme could mediate convulsant or anticonvulsant activity, although other species containing adenine are conceivable substrates e.g. cyclic 3′,5′-adenosine monophosphate, whose formation appears to be linked with transmitter release in the CNS.[9] However at present there is no reason to suppose that

TABLE 1. ACTIVITY OF β-DISUBSTITUTED GLUTARIMIDES AND THEIR SELF-ASSOCIATION IN CHLOROFORM

Drug	Activity	Concentration range (M)	Nonbonded N—H frequency (cm^{-1})	Extinction coefficient ($M^{-1}cm^{-1}$)	Association constant and range (M^{-1})
Glutarimide	Inactive	0·01,0·04	3369	142 ± 2	1·8(1·6,2·2)
β,β-Dimethyl glutarimide	Convulsant	0·01,0·04	3371	146 ± 2	2·3(2·0,2·7)
β-Methyl-β-ethyl glutarimide	Convulsant	0·01,0·08	3371	151 ± 3	2·8(2·5,3·2)
β-Methyl-β-*n*-propyl glutarimide	Dual activity	0·01,0·03	3372	149 ± 2	2·4(2·1,2·8)
β-Methyl-β-*n*-butyl glutarimide	Anti-convulsant	0·01,0·04	3372	146 ± 1	2·0(1·9,2·2)
β-Methyl-β-*n*-pentyl glutarimide	Anti-convulsant	0·01,0·04	3372	151 ± 3	2·4(1·9,3·0)

adenine is involved in these activities, and no data on the relative strength of bonding by adenine derivatives to convulsant or anticonvulsant drugs, although the potent anticonvulsant 5-ethyl, 5-phenylbarbituric acid forms a very strong complex with 9-ethyladenine, the association constant being 1200 M^{-1}.[2] In the hope of elucidating this aspect of the problem, a study of the association between 9-ethyladenine and a series of β-methyl, β-alkylglutarimides has been undertaken. The series is particularly useful, since a graded change from convulsant to anticonvulsant activity is obtained by lengthening the second β-substituent,[10] as shown in Table 1. Furthermore the β-methyl, β-ethyl derivative and several related glutarimides also inhibit the respiratory chain.[11]

MATERIALS AND METHODS

Hydrogen bonding of an N—H group alters the infrared stretching frequency and thus provides a means of measuring the extent of bonding. Equations which can be used to calculate association constants from the relative intensities of the free and associated N—H peaks have been derived by Kyogoku *et al.*,[12] for molecules which contain both a C=O and an N—H group, arranged so that two hydrogen bonds can be formed with the corresponding groups of a similar molecule.

In the specific case of a cyclic dimerization, a plot of the absorbance at the nonbonded N—H frequency, A, against C_t/A, where C_t is the total concentration, gives a straight line whose slope and intercept may be used to calculate an association constant as well as the true, rather than apparent, extinction coefficient. If only one bond is formed a straight line would not normally occur.

The same situation applies to the cyclic association of two different species, X and Y, provided that an equimolar mixture of the two species is employed, and that the dimerizations XX and YY are equally important. If a straight line plot of A against C_t/A is obtained from measurements of the nonbonded $N—H$ peak of either X or Y a cyclic association is probable. For a plot based on the appropriate peak in the spectrum of X, the association constant for XX can be used in conjunction with the values of the slope and intercept to calculate an association constant for XY, as well as the true extinction coefficient for the monomeric species, X. In this work, measurements have been made at both nonbonded $N—H$ stretching frequencies so that two independent values of each association constant could be obtained. The agreement or otherwise between these values, and between the extinction coefficients calculated from the association and dimerization experiments, constitutes a check on both the accuracy of the results and the cyclic nature of the association.

Nonbonded $N—H$ absorbance was determined from the observed spectrum by subtracting the solvent absorbance, as well as the contributions from other peaks with significant extinction coefficients at the nonbonded $N—H$ frequency. Since these contributions were often dependent on the association constant being determined, the calculations were carried out using an iterative computer program, which also incorporated a least squares fit of A against C_t/A and an evaluation of confidence limits from observed scatter.

The 9-ethyladenine used was purchased from Cyclo Chemical Co., Los Angeles, and samples of glutarimide, β,β-dimethylglutarimide, β-methyl, β-ethylglutarimide, β-methyl, β-*n*-propylglutarimide, β-methyl, β-*n*-butylglutarimide and β-methyl, β-*n*-pentylglutarimide were kindly provided by Dr. A. Shulman of the Pharmacology

Department, Melbourne University and the Nicholas Institute, Sherbrooke, Victoria. Each of these compounds was recrystallized from chloroform, which was also chosen as the solvent for the spectral studies. The latter choice meant that the interactions observed were characteristic of a relatively nonpolar environment rather than aqueous solution, although either situation might apply at the site(s) of action of the drugs. Spectroscopic quality chloroform, free of ethyl alcohol, was purchased from Mathieson, Coleman and Bell, Los Angeles.

Spectra were recorded with a Unicam SP 100 (Mark 2) spectrophotometer using a slit width of approximately 330 mμ. Time averaging of intensity levels was achieved by disengaging the frequency drive at required frequencies, or, if overlapping peaks made a continuous record of the spectrum necessary, by repeated scanning at a speed of 40 cm^{-1}/min and subsequent averaging.

To ensure constant conditions an RIIC microcell with NaCl windows, a path length of 1 mm and a capacity of 0·06 ml was used for the spectra of both solvent and solutions, which were taken at temperatures between 28° and 30°. The frequency scale was calibrated against the lines in the ν_1 band of ammonia.[13]

RESULTS

Self-associations

The distinct vibrational frequencies absorbed due to the bonded and nonbonded stretching of N—H bonds participating in hydrogen bond formation may be assigned by observing the effects of solution and concentration on their apparent extinction coefficients. Hydrogen bonding increases with concentration, so that the broad peaks

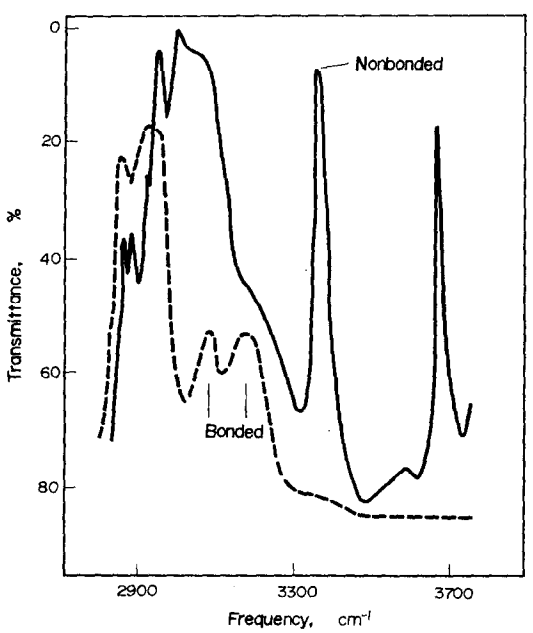

FIG. 1. Infra-red spectra of β,β-dimethylglutarimide.
————, 0·1 M solution of β,β-dimethylglutarimide in chloroform; – – – – –, β,β-dimethylglutarimide in nujol mull.

associated with bonded forms become stronger as concentration is increased. Nonbonded absorptions are correspondingly weakened, and may disappear entirely in the spectrum of the solid. The spectrum of solid β,β-dimethylglutarimide in a nujol mull, and that of a 0·1 M solution in chloroform, are shown in Fig. 1, where several qualitative differences are apparent. The bands in the solution spectrum at 3000, 3600 and 3680 cm^{-1} originate from the solvent, but the other peak missing from the solid spectrum, that at 3371 cm^{-1}, becomes weaker as the concentration is increased, and is assigned to the nonbonded N—H stretch of the monomer. On the other hand, the shoulders at 3080 and 3180 cm^{-1} are associated with the bonded N—H stretching vibrations, as they become more significant at higher concentrations and are strongest in the spectrum of the solid.

The absorbance of the peak at 3371 cm^{-1} was measured over the concentration range 0·008 M to 0·044 M, and when plotted against C_t/A gave a straight line, indicating that the dimeric complex shown in Fig. 2 is formed. The calculated association constant was 2·3 M^{-1}.

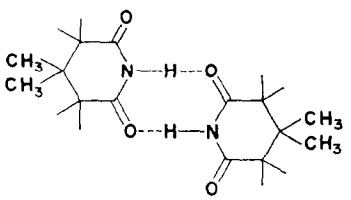

FIG. 2. Dimeric complex formed by β,β-dimethylglutarimide in chloroform solution.

The spectra of the other β-glutarimides, in nujol mull or in solution, were very similar to those of β,β-dimethylglutarimide, with the nonbonded N—H stretching frequency falling in the range 3369 cm^{-1} to 3372 cm^{-1}. In each case a straight line plot of A against C_t/A was obtained. The calculated association constants and extinction coefficients are given in Table 1, and remain almost constant throughout the series.

Several determinations of the self-association of 9-ethyladenine were made using the concentration dependence of the nonbonded N—H peak at 3410 cm^{-1}. The value obtained for the dimeric association, 4·3 M^{-1}, is a little higher than the association constant observed[12] in deuterochloroform, 3·1 M^{-1}, but not as high as the apparent value of 13 M^{-1} which has been reported[14] in chloroform containing ethyl alcohol as preservative.

Association of glutarimides with 9-ethyladenine

The spectrum of an equimolar mixture of unsubstituted glutarimide and 9-ethyladenine in chloroform over the range from 2900 to 3800 cm^{-1} is shown in Fig. 3, as well as the spectra of the individual compounds. The nonbonded N—H stretching frequencies of 9-ethyladenine at 3410 and 3520 cm^{-1}, and that of glutarimide at 3369 cm^{-1}, are also visible in the spectrum of the mixture. Bonded N—H stretching modes are responsible for the peak at 3480 cm^{-1}, and the broad shoulder in the vicinity of 3330 cm^{-1}.

The effects of hydrogen bonding could be measured using the concentration dependence of the peak at 3369 cm^{-1} for the drug, with either of the nonbonded N—H bands

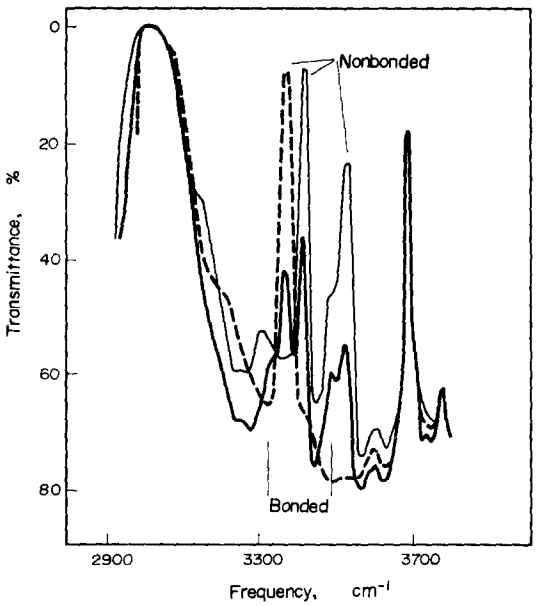

FIG. 3. Infra-red spectra of 9-ethyladenine, glutarimide, and an equimolar mixture of the two compounds.
————, 0·025 M glutarimide and 0·025 M 9-ethyladenine in chloroform; – – – – –, 0·1 M solution of glutarimide in chloroform; ————, 0·05 M solution of 9-ethyladenine in chloroform.

for 9-ethyladenine. The peak at 3410 cm^{-1} is less influenced by overlap from adjacent bands and was therefore chosen, and measurements of the dependence of A on C_t/A were made at 3369 and 3410 cm^{-1}. In both cases the experimental data were adequately accounted for by a straight line, and the association constants calculated from their slopes and intercepts were 19 M^{-1} at 3369 cm^{-1} and 24 M^{-1} at 3410 cm^{-1}. The extinction coefficients calculated from the same parameters were 147 $M^{-1}cm^{-1}$ at 3369 cm^{-1} and 228 $M^{-1}cm^{-1}$ at 3410 cm^{-1}, which compare well with the values for the individual compounds, 142 and 230 $M^{-1}cm^{-1}$ respectively. This agreement adds weight to the results, and the difference between the two calculated association constants is accounted for by the appropriate error ranges (Table 2).

Similar results were obtained for equimolar mixtures of the five β,β-disubstituted glutarimides with 9-ethyladenine, and these are summarized in Table 2. In every case straight lines were obtained for plots of A against C_t/A for both components of the mixture, and the extinction coefficients were in satisfactory agreement with those calculated earlier. In addition, the two association constants were always of similar magnitude. All of these facts point to the formation of complexes involving two hydrogen bonds between the drugs and 9-ethyladenine, which could take either of the forms shown in Fig. 4.

It is also apparent from Table 2 that the association constants for the complexes are effectively independent of the substituent groups, being in the vicinity of 20 M^{-1} for each of the drugs studied. This is greater than the self-association constants for either species, so that the formation of complexes between the drugs and 9-ethyladenine is favoured over dimerization. For example, in a 9-ethyladenine/glutarimide mixture

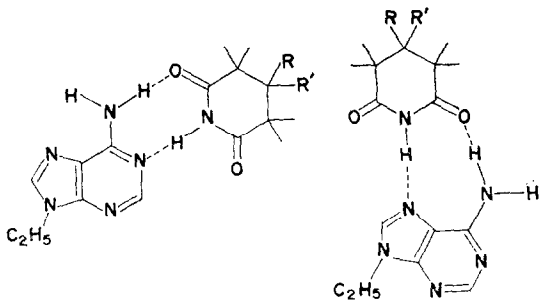

FIG. 4. Possible forms of the dimeric complex between 9-ethyladenine and β,β-disubstituted glutarimides in chloroform solution.

TABLE 2. ASSOCIATION OF β-DISUBSTITUTED GLUTARIMIDES WITH 9-ETHYLADENINE IN CHLOROFORM

Drug	Concentration range (M)	9-Ethyladenine peak, 3410 cm^{-1}		Drug peak		
		Extinction coefficient ($M^{-1}cm^{-1}$)	Association constant and range (M^{-1})	Frequency (cm^{-1})	Extinction coefficient ($M^{-1}cm^{-1}$)	Association constant and range (M^{-1})
Glutarimide	0·002, 0·012	228 ± 8	24 (17, 34)	3369	147 ± 7	19 (12, 33)
β,β-Dimethyl glutarimide	0·002, 0·010	223 ± 4	17 (13, 22)	3371	151 ± 8	26 (17, 48)
β-Methyl-β-ethyl glutarimide	0·002, 0·012	225 ± 5	22 (18, 27)	3371	152 ± 6	19 (13, 28)
β-Methyl-β-*n*-propyl glutarimide	0·002, 0·011	221 ± 4	22 (18, 26)	3372	152 ± 6	20 (13, 34)
β-Methyl-β-*n*-butyl glutarimide	0·002, 0·011	225 ± 4	19 (14, 27)	3372	148 ± 6	20 (13, 38)
β-Methyl-β-*n*-pentyl glutarimide	0·002, 0·013	216 ± 5	15 (11, 20)	3372	151 ± 7	16 (10, 28)

Two independent determinations of the association constants were obtained by employing intensity measurements at the chosen nonbonded N–H frequencies of both 9-ethyladenine and the drugs (see Materials and Methods).

with a total concentration of 0·01 M of each species, the calculated association constants give equilibrium concentrations of 0·0081 M 9-ethyladenine, 0·0003 M 9-ethyladenine dimer, 0·0083 M glutarimide, 0·0002 M glutarimide dimer and 0·0013 M 9-ethyladenine/glutarimide complex.

DISCUSSION

The constant strength of the association with 9-ethyladenine throughout the series suggests that such an association might be connected with the direct action in the respiratory chain. This is observed at 0·01 M for the convulsants β-methyl, β-ethyl-glutarimide and β-methyl, β-isopropylglutarimide, and at 0·001 M for the more liposoluble hypnotics β,β-di-*n*-propylglutarimide and α-ethyl, α-phenylglutarimide.

The adenine moiety in either nicotinamide-adenine dinucleotide or FAD could provide a suitable substrate.

On the other hand, the strength of the association is clearly unimportant in distinguishing between convulsant and anticonvulsant activity, and the results provide no indication that adenine is an integral part of the substrate(s) in either case. However they are consistent with the hypothesis[15] that a related association may be a prerequisite for convulsant or anticonvulsant action, with a second variable determining the qualitative and quantitative characteristics of the activity. The size and shape of the substituent groups could provide this variable by influencing the ability of a drug to reach a particular substrate, the overall strength of the association, and the resulting effect on substrate conformation.[16] A study of the preferred conformations of some convulsant and anticonvulsant barbiturates and glutarimides has been begun, and their interactions with FAD and other possible substrates are being investigated experimentally.

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