THE RELATIONSHIP BETWEEN THE INFRA-RED ABSORPTION SPECTRA OF SOME 5:5′-SUBSTITUTED BARBITURIC ACIDS AND THEIR PHARMACOLOGICAL ACTIVITY

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As barbiturates are used so extensively in clinical practice as depressants of the central nervous system, it is important to provide as much information as is possible about the relationship between their physicochemical and pharmacological properties. Barbiturates are classified pharmacologically on the basis of the duration of depression of the central nervous system which they produce and, therefore, representative members of the series from the long, moderate, short and ultra-short duration of action groups were selected for study.

This paper records the frequencies of the peaks of the absorption bands in the infra-red region of the spectrum associated with the carbonyl groups in each of the 14 barbiturate molecules considered.

It was thought that the various alkyl and aryl groups substituted in the 5:5′-position and the introduction of a sulphur atom in place of the oxygen atom in the 2-position might modify the double-bond single-bond resonance, $C{=}O$, $\overset{+}{C}{-}\overset{\cdot\cdot}{\underset{\cdot\cdot}{O}}\overset{-}{:}$, of the carbonyl bonds in the barbiturate nucleus to an extent which would cause changes in the frequencies of the infra-red carbonyl bands. Although the band shifts observed were small there appears to be some connection between the pharmacological action and the absorption spectra in that the shorter the duration of the action of the drug the lower were the frequencies of the carbonyl bands. The physicochemical implications arising from the analysis of the absorption bands and their relation to the carbonyl bonds are also considered below.

Method

The infra-red absorption spectra of the barbiturates were recorded in the frequency range from 1650 to 1850 wave numbers (wavelength range from 6·1 to 5·4 microns) on a rock-salt prism spectrometer. Solution strengths of about 1/100 molar in diethyl ether were found to give suitable spectra in path lengths of about 1 mm. The choice of solvent proved difficult since it is important to avoid any appreciable shift of the absorption bands, which may arise from molecular association in strongly polar solvents, if the bands are to be used to characterise the potentialities of the isolated molecule. The barbiturates studied were unfortunately

insoluble in normal non-polar solvents such as carbon tetrachloride, carbon disulphide, hydrocarbons, etc., with the exception of amylobarbitone, which was sufficiently soluble in carbon tetrachloride for a spectrum to be obtained. In order to decide whether chloroform, in which all the derivatives are very soluble, would be a sufficiently inactive solvent to use, the infra-red spectrum of amylobarbitone obtained using chloroform as a solvent was compared with that obtained in carbon tetrachloride. It was found that the absorption peaks in chloroform were very much broadened and displaced towards lower frequencies and it was therefore reluctantly concluded that there was too much solute-solvent association for chloroform to be a useful solvent. Various

TABLE I

THE FREQUENCIES OF THE PEAKS OF THE INFRA-RED ABSORPTION BANDS AND THE DURATION OF PHARMACOLOGICAL ACTION OF SOME BARBITURATES. THE SHORTER THE DURATION OF CENTRAL NERVOUS DEPRESSION THE LOWER ARE THE FREQUENCIES OF THE THREE BANDS.

Duration of action on C.N.S.		Barbiturate compound	Substituted groups		Peak of infra-red absorption band (wave numbers)			*Duration of action in rabbits of 60 per cent. of LD50 intraperitoneally	
			R	R′	*a*	*b*	*c*	hours	minutes
Long	1	PHENOBARBITONE	Ethyl-	phenyl-	1712	1746	1754	22	—
	2	BARBITONE	Ethyl-	ethyl-	1712	1739	1756	18	10
	3	RUTANOL	Methyl-	phenyl-	1717	1745	1754	—	—
	4	METHYL PHENOBARBITONE	Ethyl-	phenyl-methyl-	1695	1729	1756	—	—
Moderate	5	ALLOBARBITONE	Allyl-	allyl-	1712	1742	1755	—	—
	6	PROBARBITAL	Ethyl-	*iso*propyl-	1710·5	1741	1762	8	30
	7	APROBARBITAL	Allyl-	*iso*propyl-	1710	1740	1756	6	0
	8	AMYLOBARBITONE	Ethyl-	*iso*amyl-	1710	1742	1764	3	54
	9	SANDOPTAL	Allyl-	*iso*butyl-	1708	1741	1755	—	—
Short	10	PENTOBARBITONE	Ethyl-	l-methylbutyl-	1711	1741	1755	2	48
	11	CYCLOBARBITONE	Ethyl-	*cyclo*hexenyl-	1709	1739	1753	2	32
Ultra-short	12	HEXOBARBITONE	Methyl-	*cyclo*hexenyl-l-methyl-	1701	1722	1754	—	†42
	13	THIALBARBITONE	Allyl-	*cyclo*hexenyl-2-thio-	1708	1737		—	—
	14	THIOPENTONE	Ethyl-	l-methylbutyl-2-thio-	1708	1743		—	†28

* After Fitch and Tatum[1], Werner, Pratt and Tatum[2].
† By intravenous administration.

other solvents such as some of the higher alcohols and the chlorinated hydrocarbons were tried and found unsatisfactory. Diethyl ether was finally chosen as all the derivatives with one exception were soluble in it and the spectrum of amylobarbitone in diethyl ether was virtually the same as in carbon tetrachloride as was also true of several other non-barbiturate compounds containing the carbonyl group which were soluble both in ether and carbon tetrachloride. Anæsthetic grade ether, freed from water by standing it over anhydrous sodium sulphate, was employed and this was found to be completely transparent in thicknesses of 1 mm., over the spectral range investigated. The average spectral band width over the range of observations was about 2 wave numbers and the frequencies were determined by reference to the well-defined atmospheric bands of water vapour which fell within the same range.

RESULTS

The frequencies of the peaks of the infra-red absorption bands (*a*, *b* and *c*) associated with the carbonyl groups in the barbiturates studied are recorded in Table I together with the duration of the depression of the central nervous system in rabbits produced by the intraperitoneal administration of 60 per cent. of the LD50 of certain of the barbiturate sodium salts (Fitch and Tatum[1], Werner Pratt and Tatum[2]). Throughout this paper the order in which the barbiturates are tabulated commences with those of the longest duration of action and terminates with those with the shortest actions (Tatum[3]).

DISCUSSION

Relationship between infra-red absorption bands and pharmacological action

When the frequencies of the peaks of the absorption bands associated with the carbonyl groups are plotted for each of the barbiturates arranged in order of pharmacological activity (Fig. 1), a degree of correlation appears. In general the shorter the duration of activity, the lower are the frequencies of the 3 absorption bands. This might be associated with a slight increase in water solubility of the substance which would follow from the increased resonance of the carbonyl to $\overset{+}{C}—\overset{-}{O}$ structures indicated by such shifts. However, these changes are only minor ones and exceptions must be expected where other factors depending more

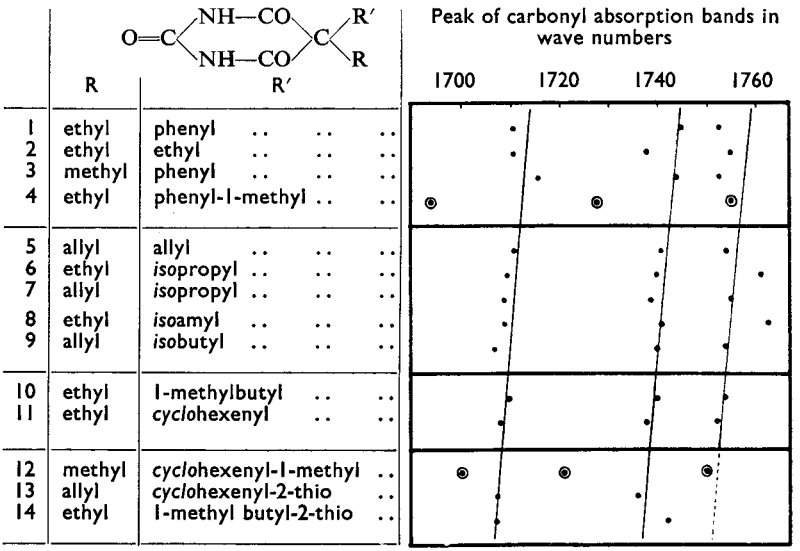


FIG. 1. The frequencies of the peaks of the infra-red absorption bands are plotted for fourteen barbiturates arranged in order of decreasing duration of depression of the central nervous system as in Table I. The peak frequencies for those barbiturates having a methyl group substituted at the 1-position are indicated by the symbol ⊙. In these barbiturates, the 2 lower of the 3 bands are displaced towards the low-frequency end of the spectrum.

upon the properties of the substituent groups themselves are involved. There are 2 types of exception to this rule. The first concerns methylphenobarbitone and hexobarbitone, each of which possesses a methyl group attached to the C atom at the 1-position. This appears to be associated with a displacement of the 2 lower of the 3 absorption bands towards the low frequency end of the spectrum. The second exception is that of the thiobarbiturates which have a C=S linkage in place of the carbonyl group in the 2-position. The physico-chemical implications of these 2 exceptions are discussed in the next section.

If the actual duration of action is plotted against the peak frequencies of the (*a*) absorption bands (Table I), the trend for the short duration of action drugs to have a lower frequency of absorption in the infra-red than the longer duration barbiturates is seen to be broken only by point 10, that in the case of pentobarbitone (Fig. 2).

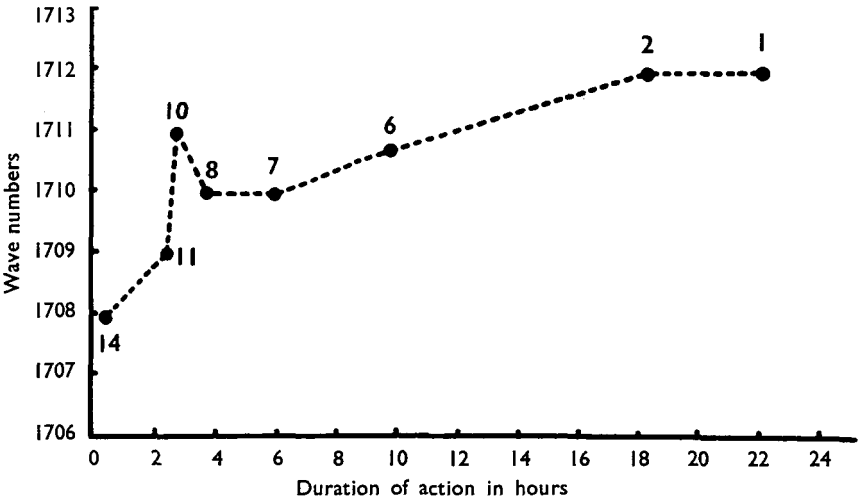


FIG. 2. This graph shows the relation between the duration of action of some barbiturates and the peak of the lowest (*a*) of the infra-red absorption bands associated with the carbonyl groups in the molecule. It is compiled from the data in Table I, and the number beside each point refers to the order of the barbiturate in Table I.

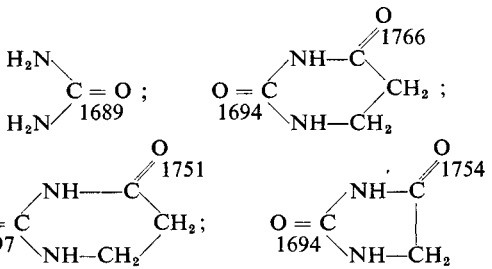
As the difference in the frequencies of the carbonyl absorption bands between members of the series is so small, the infra-red absorption spectra cannot be used to identify the individual members of the barbiturate series of drugs by consideration of the carbonyl frequencies alone. They can, of course, be identified by using the whole spectral range (Umberger and Adams[4]). Solvent difficulties may be avoided with the pressed potassium bromide disc technique. This, however, would not give the spectra characteristic of unassociated molecules which are required in order to explain differences in their chemical activity attributable to changes in the barbiturate nucleus.

In Figure 2, there are only 3 wave numbers between phenobarbitone with a duration of action of 22 hours and cyclobarbitone which acts for

2 hours 32 minutes and this indicates that extensions in the duration of pharmacological action are not to be sought in major changes in the chemical nature of the nucleus. The small frequency shifts that occur can, however, probably be linked with small changes in water solubility or lipin solubility which may be one important factor in the duration of activity of the drug.

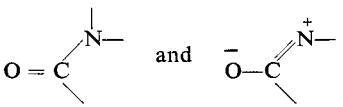
Analysis of absorption bands and their relation to the carbonyl bonds

In Figure 1, there appear to be 3 strong absorption bands in the wave number region 1700 to 1770 with each barbiturate of the long, medium and short duration of action groups. The first of these bands occurs around 1710, the second around 1740 to 1745 and the third around 1755 wave numbers. A comparison of the band positions with those of a number of structurally related molecules such as those given below (from Randall, Fowler, Fuson and Dangl[5]) leads to the conclusion

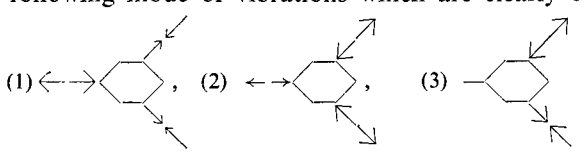


that the 1710 frequency is associated with the carbonyl in the 2-position while that in the 1755 cm.$^{-1}$ region is associated with vibrations of the carbonyl in the 4- or 6-positions. The band in the 1740 position is probably associated with a vibration which is an in-phase combination of the vibrations in the carbonyl bonds in the 2- and 4-, 6-positions.

In order to understand the vibrations of the system it should first be stated that according to modern resonance theory (Pauling, Corey and Branson[6]) the resonance in the barbiturate ring between forms containing



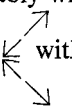
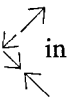
is such as to make coplanar all atoms except those in the R and R′ groups. The three carbonyl bonds act as three coupled oscillators giving rise to the following mode of vibrations which are clearly obtained by



coupling the oscillators "in phase" and "out of phase." Vibration (1) is to be associated mainly with vibrations in the carbonyl bond in the 2-position as this is expected to have the lowest bond frequency because

it has two neighbouring nitrogen atoms from which it can draw charge enabling it to have more single bond polar ($\overset{+}{C}—\overset{-}{O}$) character. In such a system the lower frequency bond largely controls the frequency and the motion is mainly in this bond. The higher frequency bonds move in phase with relatively small displacements. At higher frequencies when the vibration does correspond to motion mainly in the 4 and 6 bonds as in vibration 2, the vibration in the 2 carbonyl bond because of its lower value must follow it in opposite phase and will produce a vibration of somewhat lower frequency than that which would be associated for example, with a molecule with 4 and 6 carbonyl bonds but none in the 2-position. Thus vibration 2 is to be associated with the bands in the 1740 cm.$^{-1}$ region. It should be noted that the changing dipole associated with vibrations (1) and (2) are along the OX direction which in this case is the axis of symmetry and the motions mix with one another on this account. On the other hand, vibration (3) has its change perpendicular to the axis and therefore cannot involve motion in the 2 bond. Thus it can attain the highest frequency without modification by the lower value of the 2 bond. It is therefore to be identified with the highest frequency group around 1755 cm.$^{-1}$.

In the two substances with a methyl group attached to the nitrogen in the 1-position (viz., methylphenobarbitone and hexobarbitone) bands are obtained at lower frequencies than for any of the other compounds. In particular, it is the 2 lowest frequency bands whose frequencies are reduced, while the highest frequency band remains largely unaffected. This is consistent with the above analysis which indicates mixing with the 2 carbonyl bond for vibrations (1) and (2) but not for (3). The effect of substituting methyl groups is largely to permit greater charge transfer from the 1.N atom to the 2 and 4 carbonyl bonds thus enabling these bonds to assume greater single bond polar character. Similar frequency reductions are obtained in replacing hydrogen atoms by alkyl groups in simpler cases, e.g., the carbonyl bond in urea at 1689 cm.$^{-1}$ is shifted to 1618 cm.$^{-1}$ in *sym*-diethyl urea.

Finally we have to consider the two derivatives with C=S bonds in the 2-position. This bond has a much lower frequency (*ca.* 860 cm.$^{-1}$) than that of a carbonyl and thus does not interact appreciably with them and we are left with two carbonyl vibrations one in phase  with dipole change along the axis of symmetry and one  in which the change is perpendicular to this axis. Another important factor is that, since the C=S bond has much less electronegativity than has the C=O bond there is practically no drain of negative charge from the 1 and 3 nitrogen atoms to the 2-position in these substances and this charge is therefore released to go to the carbonyl bonds in the 4 and 6-positions. The result is that the bands corresponding to vibrations of these bonds occur

at lower frequencies than do the corresponding bands in sulphur-free barbiturates. This also indicates that the 4:6-carbonyl bonds in the thiobarbiturate compounds are more strongly polar than the same bonds in non-sulphur barbiturates containing molecules.

The correlation of the pharmacological activity with the condition of the carbonyl bond as judged from Figure 1, would indicate that the greater the double bond character of the carbonyl groups, the higher the bond frequencies, the lower the polarity, the longer the duration of activity. The differences between the different drugs are, however, small and the activity appears to depend on other factors in addition. It does not seem that the various groups R and R′ can exert much more than a steric effect. Of probably more importance is their capacity to increase the fat solubility, an effect which would be expected also to increase with increase in double bond character (reduction in polarity). This latter effect may well be the important factor which controls the duration of activity by permitting longer retention of the intact barbiturate ring in the body. The infra-red evidence presented here, though intrinsically interesting, and indicating features which should result in somewhat greater fat and lower water-solubility of the active nucleus for the longer acting drugs, does not suggest any other major factors which might affect the duration of their activity.

Summary

1. The infra-red spectra of a number of barbiturates classified according to the duration of their activity in depressing the central nervous system have been recorded in the range of the carbonyl frequencies.

2. An analysis of the bands obtained has been made and their relation to the bonds derived.

3. It is found that the longer acting drugs tend to have a band system at slightly higher frequencies.

4. This indicates slightly lower polarities and greater fat-solubility, which is suggested as being a possible contributory cause to the duration of their activity.

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