Determination of Barbituric Acid Derivatives as Mercury Compounds III. Composition and Structure of Isolated Compounds

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In the compounds between mercury and barbituric acid derivatives, which have been used for the determination of barbiturics, the mercury is attached to the unsubstituted nitrogen atoms with homopolar bonds. One atom of mercury combines with one molecule of a barbituric acid derivative, which has two free NH groups. The resulting compounds are polymers with high molecular weights. One atom of mercury combines with two molecules of a barbituric acid derivative, which has one free NH group. The resulting compounds are monomers.

A new sensitive and quite specific method for the determination of barbituric acid derivatives has recently been developed (Refs.¹⁻³). The compounds are extracted together with an equivalent amount of mercury into an organic solvent, and the mercury determined. Mercury(II) salts have been used as precipitating agents for barbiturics in several other methods — both qualitative and quantitative ones — and the compositions of the precipitates have been given (Refs.⁴⁻⁶). However, all these methods are concerned with aqueous solutions, and the reaction products may change when taken up in an organic solvent. In this paper the structures of the compounds formed in the analytical procedure have been investigated.

RESULTS AND DISCUSSION

As seen in Table 1 the compounds can be divided into two different groups, one consisting of barbituric acids which are di-substituted at the carbon atom number 5, and the other of acids also substituted at a nitrogen atom. The composition of the mercury compounds of the first group, corresponds closely to a compound, where in one molecule of barbituric acid two hydrogen atoms have been substituted for one atom of mercury. In the second group one atom of mercury substitutes a hydrogen atom in each of two molecules of barbituric acid (Table 3).

Table 1. Barbituric acid investigated.

Code used in text	R	R ₁	R'	R''
A	C ₂ H ₅	<i>i</i> -C ₅ H ₁₁	H	H
B	C ₂ H ₅	C ₆ H ₅	H	H
C	C ₂ H ₅	<i>neo</i> -C ₅ H ₁₁	H	H
D	C ₂ H ₅	C ₆ H ₅	CH ₃	H
E	C ₂ H ₅	<i>neo</i> -C ₅ H ₁₁	CH ₃	H
F	C ₂ H ₅	<i>neo</i> -C ₅ H ₁₁	CH ₃	CH ₃
G		CH ₃	CH ₃	H

It should be emphasized that none of the mercury compounds investigated contained any appreciable amount of foreign matter, *e.g.* chloride, which might have been introduced from the chloroform. Some of the substances made by former authors contained negative groups, *e.g.* chloride, nitrate, hydroxyl etc.

From Table 2 it may further be concluded that the mercury compounds have molecular weights corresponding to mercuri-(N-methyl-barbituric acid)₂ and (mercuri-barbituric acid)_n, where the subscript *n* has a value of about 100. The degree of polymerisation seems to depend on the mode of isolation.

There are some additional differences between the two types of mercury compounds. Those derived from the N-methyl-barbituric acids are more soluble in chloroform than the other category and separate as a fine powder.

Table 2. Molecular weights.

Mercury compound of substance		Boiling point method	Light scattering method	Theory
A	Amobarbital	Too great to be measured	—	(426) <i>n</i>
C	Nevental®			
	Preparation 1	Too great to be measured	50 000 ± 10 %	(437) <i>n</i>
	2	» » » » »	30 000 ± 10 %	
	3	» » » » »	32 000 ± 10 %	
	4	» » » » »	51 000 ± 10 %	
E	N-Methyl-Nevental®	781; 938; 836; 710, M = 814	To small to be measured	703
G	Hexobarbital	701; 655; 660; 625, M = 660	To small to be measured	671

Table 3. Analyses of mercury compounds of the substances C, G, E.

	C Nevental ®			G Hexobarbital			E N-Methyl-Nevental ®		
	Per cent			Per cent			Per cent		
	Found	Calc. for $C_{12}H_{16}O_3N_2Hg$		Found	Calc. for $(C_{12}H_{15}O_3N_2)_2Hg$		Found	Calc. for $(C_{13}H_{19}O_3N_2)_2Hg$	
C	32.60	32.70	32.99	43.25; 42.93; 43.05	42.95	44.39; 44.78; 44.84	44.41		
H	3.98	4.02	3.69	5.44 5.01 5.50	4.51	5.76 5.80 5.76	5.45		
N	6.38	6.32	6.41	8.34 8.56 8.39	8.35	7.73 7.72	7.97		
Cl	0.04	0.02	0.00			0.8 0.8	0.00		
P or S	P: 0.08	0.07	0.00	S: 0.00 0.00	0.00				
Hg	44.85	44.66	45.92	29.79 30.09	29.89	27.83	28.53		
Barb. ac.	54.2	54.4	54.08	70.13	70.11	70.0 71.2	71.47

Fig. 1. IR absorption of Neven tal (C) (KBr phase).

Fig. 2. IR absorption of deuterated Neven tal (C) (KBr phase).

The high molecular compounds are obtained as a glass. When dry the difference in the rate of dissolution in chloroform is very great. It must be kept in mind that all investigations refer to the substances in chloroform solutions or in the solid state.

Infrared spectra. The characteristic infrared absorption of barbiturates has been studied by several authors. Umberger and Adams ⁷ identified barbiturates from their IR spectra in chloroform solution. Price *et al.*⁸ correlated the pharmacological properties of some barbiturics with their IR absorption in the carbonyl region. Manning and O'Brien ⁹ proved the value of the method using various group frequencies in the molecules for identification purposes. Recently, Danielsson and Sandberg ¹⁰ pointed out the difficulties in classifying N- and N,N'-substituted barbituric acids by their infrared absorption. Levi and Hubley ¹¹ identified barbiturates as copper(II)-pyridine complexes in Nujol and in potassium bromide and related spectral data to structural features.

Table 4. Stretching frequencies in the NH and CO regions.

Code	Barbituric acid					Hg(II) compound				
	NH		CO			NH		CO		
A	3220	3100	1763	1732	1702	—	—	1698	1632	
B	3310	3200	3110	1777	1718	—	—	1701	1637	1541
								1700	1632	
C	3200	3110	1767	1718	(1683)	—	—	1707	1636	(1560)
								1704	1633	
D	3190	3100	1767	1708	(1696)	—	—	1704	1635	(1534)
								1727	1667	
E	3220	3100	1764	1718	1694	—	—	1728	1670	1631
								1729	1672	
F	—	—	1749	1723	1673	—	—	1730	1670	1634
								—	—

Fig. 3. IR absorption of Methylphenobarbital (D) (KBr phase).

Fig. 4. IR absorption of Hg-Nevental (Hg-C) (KBr phase).

As mainly the NH and CO vibrations of the barbiturate ring are involved in the bond formation with metals, the present discussion is limited to the regions around 3000 and 1700 cm^{-1} , where well-defined absorption bands of these groups appear. The non-N-substituted barbituric acids A–C display the characteristic N–H absorptions around 3200 and 3110 cm^{-1} , which are attributed to the hydrogen-bonded NH groups. Figs. 1 and 2 show the complete spectra of substance C in its normal and in its deuterated state. In the latter case the expected N–D stretching is found at 2360 cm^{-1} . Substance B exhibits an extra band at 3310 cm^{-1} presumably due to free N–H stretching vibrations¹¹. The N-methylated barbiturates D and E are characterized by a reduced intensity in the N–H absorptions (Fig. 3), and logically the N,N'-dimethyl substituted compound F has no absorption in the N–H stretching region.

From Table 4 it is evident that no mercury compound of the barbiturates A–E absorbs in the N–H region. On the other hand, among the corresponding sodium and barium barbiturates only the N-methylated compounds have lost their N–H absorption. Compared with the free non-N-methylated acids, the N–H intensities of their sodium and barium salts are reduced to the same degree, indicating *one* unsubstituted N–H group for both kinds of salts.

Of the three different peaks found the lowest frequency is associated with the carbonyl in the 2 position as this may partly assume a single bond character⁸. The two high frequencies are assigned to combined motions where the carbonyl bonds in the 4 and 6 positions are involved. The spectra of the barbiturates A–E comply with these findings. It is interesting that the band at 1720 cm^{-1} has disappeared for the N,N'-methylated compound F whereas the absorption at 1690 cm^{-1} has increased. The inductive effects of the two methyl substituents affect the carbonyl group towards single bond character especially in the 2 position. The two lowest frequencies can no longer be resolved and therefore result in one strong peak.

In the mercury barbiturates we get a drastic shift of the carbonyl stretching frequencies towards low wavenumbers (Table 4) and combined with the disappearance of the N—H stretching frequencies for the compounds A—C this is a strong support for the assumption of nitrogen-mercury bonds. For some metal-urea complexes forming nitrogen-metal bonds Penland *et al.*¹² have shown that a blocking of the resonance between one nitrogen atom and the carbonyl group will cause an increased CO double bond character. Such an explanation is not valid for the mercury barbiturates which have a low frequency band around 1635 cm^{-1} as the dominating absorption in the carbonyl region. The disubstituted acid F has an absorption pattern in the carbonyl region similar to the mercury compounds of A, B, and C but the latter compounds have a greater frequency shift. From this analogy we can conclude that the contribution of single polar bonding in the carbonyls, may partly have an analogous origin.

A methyl group and a mercury atom both exert a negative inductive effect on an adjacent nitrogen which will therefore gain an excess of negative charge. As a net result the carbonyls in the barbiturate ring are weakened and acquire more single polar bond structures. Gierer¹³ has observed that the C=O frequency in N-mono-substituted amides is sensitive to the inductive effect of the N-substituent. Thus, he found that the carbonyl frequency in N-chloroacetamide at 1705 cm^{-1} shifted to 1580 cm^{-1} for the corresponding N,N'-mercuri-bis-acetamide.

The absorption spectrum of the mercury compound of substance C is shown in Fig. 4. This compound and its spectrum are typical for the group "A—C" with mercury covalently bonded to both nitrogen atoms.

As a first approximation the inductive forces over both the nitrogen atoms may act in an additive way upon the carbonyl in the 2 position, giving an increased single bond character to this group compared to the other two carbonyls which can be regarded as equivalent. Thus, the dominating low frequency band around 1635 cm^{-1} may be associated with vibrations in the carbonyl bond in the 2 position. To a certain degree this absorption can also be correlated with an in-phase combination of the vibrations of the carbonyls. Further, the combined vibration of the carbonyls in positions 4 and 6 can only occur perpendicularly to the axis of symmetry of the molecule, thus causing the highest frequency band to appear around 1700 cm^{-1} . Although the main influence on the carbonyl groups seems to be caused by the inductive effect of the mercury atoms, an additional effect caused by coordination has to be considered. Mercury has here the coordination number 6 and if a suitable configuration can be established it is possible for the metal to interact with oxygen in adjacent carbonyl groups with coordinative forces. Thus, nothing can be concluded in this respect from the infrared spectra.

The mercury compounds with the barbiturates D and E both contain one methyl group and one mercury atom attached to nitrogen. From Fig. 5a it is obvious that these spectra are distinguished by a splitting of the strongest peak in the carbonyl region, giving absorptions at about 1730 , 1670 and 1630 cm^{-1} . This effect is more pronounced for a spectrum run in Nujol mull (Fig. 5b).

The spectra of the two mercury compounds and their corresponding barbituric acids D and E (Figs. 3, 5a and 5b) show the same general carbonyl

Fig. 5a. IR absorption of Hg-Methylphenobarbital (Hg-D) (KBr phase).

Fig. 5b. IR absorption of Hg-Methylphenobarbital (Hg-D) (Nujol suspension).

absorption. We may explain the splitting of the strongest absorption peak as above mainly by the inductive effects in the barbiturate ring. An N-substituted methyl group alone or together with a mercury atom will cause an asymmetry in the ring vibrations.

In the mercury compounds of D and E the low frequency band at 1630 cm^{-1} is little affected by the replacement of a mercury atom for a methyl group. This band is therefore assigned to the vibration of the carbonyl in the 2 position. As the symmetry of the barbituric ring is destroyed the other carbonyls in the 4 and 6 positions are no longer equivalent and give separate bands. The highest frequency at 1730 cm^{-1} may then be assigned to the 6 position carbonyl adjacent to the methyl substituted nitrogen. Finally, the vibration at about 1670 cm^{-1} has to be correlated with the CO group in the 4 position, as here the inductive effects and the coordinative influence are more pronounced because of an adjacent mercury atom.

The carbonyl region for the sodium and barium barbiturates may be interpreted similarly. These compounds contain one equivalent of metal per molecule whether one of the nitrogen atoms is methylated or not. Their car-

Fig. 6. IR absorption of Ba-Amobarbital (Ba-A) (KBr phase; before heating the disk).

Fig. 7. IR absorption of Ba-Amobarbital (Ba-A) (after heating the disk at 110°C for 10 h)

bonyl absorptions are all very similar exhibiting three distinct peaks in the region from 1520 to 1730 cm^{-1} (Figs. 6–9).

In solution and in the solid state the ionic structures I and II contribute to the electron distribution¹⁴ (Fig. 10). For a methyl substituted compound we have the analogous resonance structure III. Table 5 shows that the position of the band with the highest wave-number is constant, shifting within the range 1710 to 1727 cm^{-1} , which indicates a C=O band.

It is more difficult to explain the second and third absorption bands in the carbonyl region. The second band varies within a relative wide range (1621 to 1670 cm^{-1}) and may be attributed to a conjugated C=N bond frequency. On the other hand, changes in the environment of a C=N linkage may cause considerable frequency shifts. Nor is it possible to find any systematic change in the frequency of the second band in this case. The third low frequency band may be attributed to a carbonyl vibration in an enolate group $=\text{C}-\text{O}^{(-)}\text{M}^{(+)}$. A support for such an explanation is that the sodium barbiturates form a high frequency group (1570 to 1574 cm^{-1}) and the barium barbiturates a low frequency set (1520 to 1540 cm^{-1}) when only the third band is considered. The positions of the three bands just discussed are only slightly influenced by deuterization as seen from Table 5 and Fig. 8. Hence, the N—H bond cannot participate in a band in this region. In conclusion, we refrain

Fig. 8. IR absorption of deuterated Ba-Nevental (Ba-C) (KBr phase).

Fig. 9. IR absorption of Ba-Methylphenobarbital (Ba-D) (KBr phase).

from assigning the three absorption peaks to specific groups of the molecules and prefer to identify them with the resonance structures I, II, and III.

Further work with other metals may throw more light on this question.

EXPERIMENTAL

Preparation of mercury compounds. Several modifications of the analytical method were tried until finally the following simple procedure was adopted for preparative work. One gram of the barbituric acid derivative was mechanically shaken with 4 g of mercury(II) oxide (yellow), 20 ml of a 0.05 M sodium carbonate buffer solution with a pH of about 9.5, and 300 ml of chloroform for a couple of hours. The chloroform layer was separated, filtered and evaporated to a volume of about 20 ml. The mercury compound was then precipitated with several hundred milliliters of petroleum ether (b.p. 40–60°). The precipitate was collected, washed with petroleum ether, and dried in vacuum.

For the preparation of the deuterium barbituric acid mercury compound an aliquot of the chloroform solution, corresponding to 100 mg of barbituric acid, was evaporated until only a few ml of chloroform was left. Carbon tetrachloride was added and the

Fig. 10.

Table 5. Stretching frequencies in the NH and CO regions.

Code	Na compound					Ba compound				
	NH		CO			NH		CO		
A	3220	3090	1710	1667	1570	3210	3090	1712	1653	1533
B	3220	3100	1710	1670	1574	3210	3090	1716	1665	1540
C	3240	3110	1717	1663	1590*	3220	—	1727	1637	1522
						3210	—	1724	1635	1520
						—	—	1720	1630	1517**
D	—	—	—	—	—	—	—	1717	1634	1527
E	—	—	—	—	—	—	—	1715	1632	1523
								1715	1621	1520

* Ca-compound.

** Mainly deuterized product.

solution evaporated to a small volume. This was repeated twice in order to remove all traces of chloroform. The resultant solution, about 20 ml, was shaken for half an hour at room temperature with 10 ml of deuterium oxide, and the mixture evaporated at 0.1 mm Hg. From the introduction of the deuterium oxide, atmospheric moisture was carefully excluded.

Deuterium barbituric acids were prepared by boiling 50 mg of the barbituric acid with 10 ml of deuterium oxide. The solution was then evaporated at 0.1 mm Hg. These procedures give compounds where NH is mainly exchanged for ND.

Analyses. For the determination of mercury an amount of substance corresponding to about 20 mg of mercury was dissolved in 5 ml of alcohol and 1 ml of 5 M hydrochloric acid. 15 ml of 0.02 N EDTA was added and then 5 ml of 5 M ammonia. After the addition of 2 ml of buffer, pH = 10 (ammonia 4.5 M, ammonium chloride 1 M), the solution was titrated with 0.02 N zinc sulphate solution using Eriochrome black as indicator.

For the determination of barbituric acid in the mercury compound an amount corresponding to about 150 mg of barbituric acid was mixed with 5 ml of water and hydrogen sulphide was bubbled through for 5 min. The mixture was shaken for 10 min, 2 ml of 5 N sulphuric acid added and then extracted four times with chloroform, 15 ml each time. The chloroform was filtered through anhydrous sodium sulphate and evaporated. The residue was dissolved in 10 ml of dimethylformamide and titrated with 0.1 N potassium methylate against thymol blue. A blank was run on the dimethylformamide.

The molecular weights of some of the mercury compounds were determined by a boiling point elevation method, using a Gallenkamp apparatus¹⁵. In this instrument the temperature increase is determined by a thermistor used in conjunction with a Wheatstone bridge.

The boiling vessel was charged with 10 ml of alcohol-free, dry chloroform, containing about 100 mg of glyceryl monostearate, which makes the boiling smooth. When boiling equilibrium had been attained, the galvanometer was brought to zero position and weighed amounts (50–100 mg) of the barbituric acid derivative was introduced into the vessel. It was necessary to use the substances as loosely compressed tablets as otherwise some powder adhered to the neck of the boiling vessel. The galvanometer was again brought to zero, another tablet introduced, etc. In this way a calibration curve, a straight line through origo, was obtained, and from experiments with the mercury compounds the results in Table 2 were obtained. The precision was, however, much lower when working with the mercury compounds possibly due to the long equilibration time.

Some determination of the molecular weights were made by light scattering measurement with a Brice-Speiser light scattering photometer¹⁶. Freshly distilled chloroform was used as solvent and the solutions were clarified by filtering through a Jena G5 sintered glass filter directly into the light scattering cell. The turbidities of a stock solution (about 2 %) and three dilutions were measured at 436 m μ . The refractive index increment for this system was determined with a Rayleigh interferometer and amounted to 0.085 ml/g.

The measurements on the mercury compound of substance C were made on four different solutions.

(1) The compound was prepared by the method given on page 1489, isolated, dried, etc. and then dissolved in the chloroform.

(2) The compound was prepared as above but never isolated. Instead, the chloroform extract containing the compound was concentrated *in vacuo* until the solution contained about 2 per cent of the compound. Part of the solution was analysed and the actual concentration determined and the rest was used for the light scattering experiments.

(3) The compound was prepared as in the preceding paragraph, with the exception that the chloroform extract was concentrated on the waterbath to a 2 % solution.

(4) The compound was prepared as in the preceding paragraph, but the chloroform extract was evaporated to dryness on the water-bath, and the residue was then immediately redissolved in chloroform.

Infrared spectra. A Unicam SP 100 spectrophotometer with sodium chloride optics was used. The solid substances were examined with the potassium bromide disc technique, or in Nujol suspension. In some cases, when a sufficient concentration could be attained, the spectra were also run on chloroform solutions.

Like Jensen¹⁷, we have found some differences between spectra for barbiturates and their salts in Nujol and in potassium bromide tablets. A broad band around 3500 cm⁻¹, indicating an associated O—H stretching frequency, often appears with the disc technique but never with Nujol. Figs. 6 and 7 show that heating the disc at 110°C for some hours eliminates this band. The other parts of the spectrum are little influenced by the heating.

On the other hand, the spectra of the mercury barbiturates often change on heating — the positions of some of the bands may shift by 10 wavenumbers, and the resolution is generally improved.

The Tables 4 and 5 show values obtained on heated discs when the substance did not change at 110°C. When there was a difference between the wavenumbers for treated and untreated discs the absorption was also measured on a Nujol suspension (the values on the second line for each substance in the tables).

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