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# Combined gas-liquid chromatography—mass spectrometry in the study of barbiturate metabolism

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Combined gas-liquid chromatography-mass spectrometry is shown to be a useful technique for the identification of barbiturates and their metabolites, when applied to *NN'*-dimethyl derivatives.

Mass spectrometry offers a means of investigating the structures of organic compounds which are available in only small amounts (Budzikiewkz, Djerassi & Williams, 1964; McLafferty, 1967), hence its utility in the study of drug metabolism (Kabasakalian, Taggart & Townley, 1968, Neal, 1968; Schwartz, Vane & Postma, 1968). Combination with a gas-liquid chromatograph enables useful mass spectra to be obtained from components of a mixture which are present in sub-microgram quantities, since manipulative losses are reduced to a minimum (Holmes & Morrell, 1957). It was decided to evaluate the usefulness of this technique in determining the structure of metabolites of barbiturate drugs by ascertaining the information which could be derived from barbituric acid derivatives of known structure.

Although barbiturates themselves are amenable to gas liquid chromatography, it was found that greatly improved results were obtained when the *NN'*-dimethyl derivatives were used (Neville, 1970). This did not affect interpretation of the mass spectra. The molecular separator working on the opposed jet principle (Ryhage, 1964) gave superior results with polar compounds to the fritted glass type (Watson & Biemann, 1964) and hence was used in the investigation.

## EXPERIMENTAL

The *NN'*-dimethyl derivatives of the barbiturates were prepared by addition of an ethereal solution of diazomethane to a methanolic solution of the barbiturate. After 15 min the solution was evaporated and a 10% solution of the derivative in methanol was prepared for GLC injection in 0·5 $\mu$l quantities. GLC analysis showed 85–90% conversion to the *NN'*-dimethyl derivative. A 5 foot stainless steel column packed with 2% SE 52 on Embacel 60/100 was used, and the temperature was maintained at either 175° or 200°. The molecular separator was contained in the GLC oven. The inlet line to the mass spectrometer source was kept at 200°. The mass spectrometer was an A.E.I. M.S. 902, running at a source temperature of 220° and a beam energy of 70 eV. Resolving power was 1000 (10% valley definition). The inlet pressure of the helium carrier gas was 2·5 p.s.i. and flow rate 50 ml/min. The column, separator and mass spectrometer inlet system were pretreated with 50 $\mu$l of hexamethyldisilazane.

The formula of ions encountered in the fragmentation schemes outlined in the discussion was confirmed by accurate mass measurements at a resolving power of 20 000 (10% valley definition). Although for a given GLC peak, a single accurate mass measurement could be made on a compound passing through the system, for multiple determinations it was more convenient to make such measurements on samples admitted via the direct inlet system of the mass spectrometer.

## RESULTS AND DISCUSSION

Table 1 lists the compounds and their relative retentions, Table 2 their mass spectra.

Those compounds with saturated alkyl side chains (I–IV), and in addition *NN'*-dimethylcyclobarbitone (VI) do not give observable molecular ions in their mass spectra. This is because of the ease of elimination of a molecule of ethylene from the molecular ion via a McLafferty rearrangement (McLafferty, 1959) in those compounds (I–IV) where an ethyl side-chain is present. Although (VI) has such an ethyl side-chain, this is in the $\beta$-position to the double bond of the cyclohexenyl substituent, hence fission of the ethyl group without transfer of hydrogen (Reed, 1966) is as

Table 1. *Compounds examined and their relative retention times*

| Compound | $R^1$ | $R^2$ | Relative retention 175° | 200° |
|---|---|---|---|---|
| *NN'*-Dimethylbarbitone (I) | Et | Et | 0·48 | 0·6 |
| *NN'*-Dimethylbutobarbitone (II) | Et | $Bu^n$ | 0·78 | 0·82 |
| *NN'*-Dimethylpentobarbitone (III) | Et | $Me{\cdot}CH_2{\cdot}CH_2{\cdot}CH(Me)$- | 1·0 | 1·0 |
| *NN'*-Dimethylamylobarbitone (IV) | Et | $Me{\cdot}CH(Me){\cdot}CH_2{\cdot}CH_2$- | 0·87 | 0·89 |
| *N*-Methylhexobarbitone (V) | Me | Cyclohexenyl- | 2·09 | 1·77 |
| *NN'*-Dimethylcyclobarbitone (VI) | Et | Cyclohexenyl- | 2·55 | 2·04 |
| *NN'*-Dimethylphenobarbitone (VII) | Et | Ph | 2·43 | 1·9 |
| *NN'*-Dimethylquinalbarbitone (VIII) | $CH_2{:}CHCH_2$- | $Me{\cdot}CH_2{\cdot}CH_2CH(Me)$- | 1·18 | 1·1 |
| *NN'*-Dimethyl-5-(1-methylbutyl)-5-methoxycarbonylmethybarbituric acid (IX) | $-CH_2{\cdot}CO_2{\cdot}Me$ | $Me{\cdot}CH_2{\cdot}CH_2{\cdot}CH(Me)$- | | 1·81 |
| *NN'*-Dimethylnealbarbitone (X) | $CH_2{:}CHCH_2$- | $Me_3C{\cdot}CH_2$- | 0·9 | 0·9 |
| *NN'*-Dimethyl-3'-hydroxybutobarbitone (XI) | Et | $Me{\cdot}CH(OH){\cdot}CH_2CH_2$- | | 1·45 |
| *NN'*-Dimethyl-3'-hydroxypentobarbitone (XII) | Et | $Me{\cdot}CH(OH){\cdot}CH_2{\cdot}CH(Me)$- | | 1·57 |

expected the dominant process in this case. The other alkyl substituent present in compounds I–IV, and the hydroxylated alkyl substituents present in compounds XI and XII are able to take part in a McLafferty rearrangement, whereby they are eliminated, but with transfer of a hydrogen atom to the residual charge-carrying portion of the molecule. This gives in all cases an ion of m/e 184 which decomposes further as in Scheme 1: the fragmentation of *NN'*-dimethylpentobarbitone. These two McLafferty rearrangement processes are therefore of great diagnostic value in determining the nature of the substituents attached to the 5-position of the barbiturate ring.

*N*-Methylhexobarbitone (V) and *NN'*-dimethylcyclobarbitone (VI) behave similarly in the mass spectrometer. Just as VI loses the ethyl group by $\beta$-fission, so does V lose its methyl substituent. The cyclohexyl substituent in both cases is involved in a McLafferty rearrangement, being eliminated as a $C_6H_8$ moiety to give ions of m/e 170 and 184 respectively. *NN'*-dimethylphenobarbitone (VII) is able to lose a molecule of ethylene from the ethyl substituent in the same manner as the previous compounds. An $(M-C_6H_5)$ ion is not observed, but the presence of a phenyl substituent can be inferred by the presence of a $C_6H_5^+$ ion at m/e 77, and ions at m/e 51 and 39, typical of phenyl-substituted compounds.

*NN'*-Dimethylquinalbarbitone (VIII), loses the 1-methylbutyl side-chain from the molecular ion as the neutral olefin, to give the base peak of the spectrum, at m/e 196. The molecular ion is also able to lose the allyl group to give an ion of m/e 225. *NN'*-Dimethylnealbarbitone (X), behaves similarly.

Table 2. *Mass spectra of the compounds examined.* m/e values are followed by relative intensities in parentheses. Only those peaks with relative intensities greater than 2 for m/e above 150 and relative intensities greater than 5 for m/e below 150 are included.

NN′-*Dimethylbarbitone* (I)
212 (1), 185 (9), 184 (97), 183 (30), 170 (9), 169 (100), 156 (2), 155 (3), 126 (25), 112 (15), 98 (5), 69 (7), 58 (7), 55 (10), 41 (12).

NN′-*Dimethylbutobarbitone* (II)
212 (1), 211 (10), 210 (8), 185 (8), 184 (63), 183 (12), 170 (16), 169 (100), 156 (2), 155 (3), 154 (2), 126 (11), 112 (21), 97 (7), 83 (7), 69 (7), 58 (13), 55 (21), 42 (7), 41 (24), 39 (10).

NN′-*Dimethylpentobarbitone* (III)
226 (8), 185 (11), 184 (88), 183 (12), 170 (9), 169 (100), 156 (3), 155 (3), 126 (9), 112 (13), 97 (9), 69 (12), 58 (10), 55 (12), 53 (5), 43 (25), 41 (34).

NN′-*Dimethylamylobarbitone* (IV)
227 (7), 226 (8), 211 (5), 185 (18), 184 (81), 183 (12), 170 (22), 169 (100), 156 (2), 155 (3), 126 (12), 112 (19), 97 (8), 83 (7), 69 (12), 58 (15), 56 (22), 43 (15), 41 (33), 39 (12).

N-*Methylhexobarbitone* (V)
250 (9), 236 (16), 235 (100), 178 (7), 171 (23), 170 (18), 169 (16), 137 (9), 136 (11), 135 (5), 112 (6), 108 (11), 93 (14), 91 (18), 81 (62), 80 (20), 79 (29), 77 (22), 67 (10), 66 (7), 65 (12), 58 (23), 56 (14), 55 (8), 53 (19), 52 (10), 51 (11), 41 (30), 39 (27).

NN′-*Dimethylcyclobarbitone* (VI)
236 (15), 235 (100), 185 (2), 184 (2), 183 (2), 179 (2), 178 (8), 170 (3), 169 (29), 150 (3), 149 (2), 121 (5), 112 (6), 91 (6), 81 (9), 79 (10), 77 (7), 58 (5), 53 (5), 41 (8), 39 (6).

NN′-*Dimethylphenobarbitone* (VII)
260 (7), 245 (4), 233 (15), 232 (100), 203 (4), 188 (9), 175 (20), 147 (4), 146 (24), 118 (22), 117 (22), 115 (7), 103 (10), 91 (8), 89 (6), 77 (8), 58 (6), 51 (4).

NN′-*Dimethylquinalbarbitone* (VIII)
266 (4), 248 (4), 237 (5), 225 (5), 224 (7), 223 (6), 209 (5), 197 (12), 196 (100), 195 (72), 183 (8), 181 (25), 169 (10), 138 (22), 126 (4), 112 (7), 111 (26), 110 (14), 109 (5), 97 (10), 82 (10), 81 (9), 80 (9), 58 (17), 55 (16), 53 (20), 43 (36), 41 (54), 39 (19).

NN′*Dimethyl*-5-(1-*methylbutyl*)-5-*methoxycarbonylmethylbarbituric acid* (IX)
298 (0·3), 267 (2), 229 (2), 228 (14), 227 (1), 225 (2), 197 (5), 196 (100), 195 (2), 183 (2), 169(10), 112 (5), 43 (8), 41 (7).

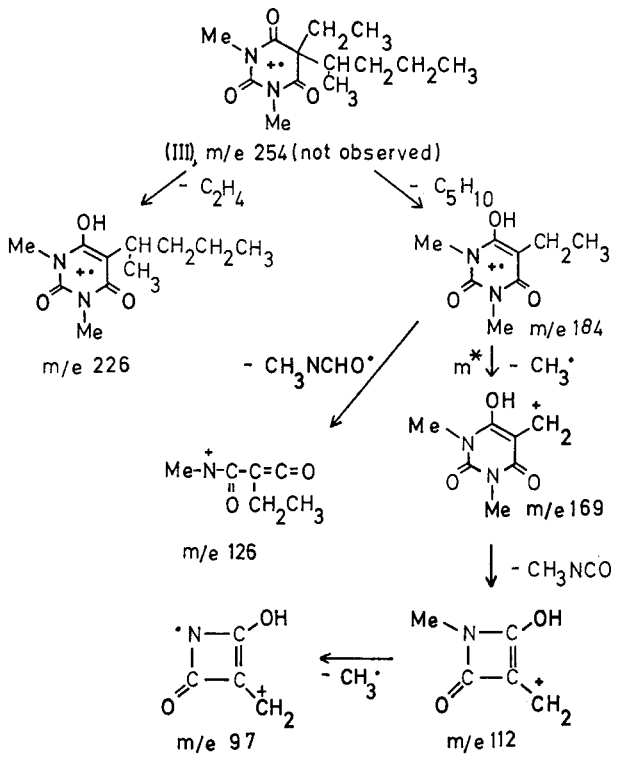
NN′-*Dimethylnealbarbitone* (X)
266 (4), 251 (21), 233 (3), 225 (5), 210 (15), 209 (64), 196 (15), 195 (80), 181 (5), 170 (13), 169 (100), 168 (8), 152 (6), 138 (14), 112 (20), 83 (10), 67 (13), 58 (12), 57 (65), 56 (8), 55 (18), 43 (13), 41 (60).

NN′-*Dimethyl*-3′-*hydroxybutobarbitone* (XI)
241 (2), 239 (2), 228 (2), 227 (6), 211 (3), 210 (3), 209 (12), 195 (2), 186 (4), 185 (28), 184 (40), 183 (6), 171 (5), 170 (14), 169 (82), 157 (5), 156 (4), 155 (10), 154 (4), 152 (4), 141 (5), 126 (22), 112 (32), 97 (24), 83 (19), 81 (11), 70 (16), 69 (34), 68 (15), 67 (18), 58 (72), 57 (21), 56 (29), 55 (64), 54 (23), 53 (34), 45 (100), 44 (17), 43 (84), 42 (36), 41 (68), 40 (18), 39 (52), 31 (19), 30 (16), 29 (60).

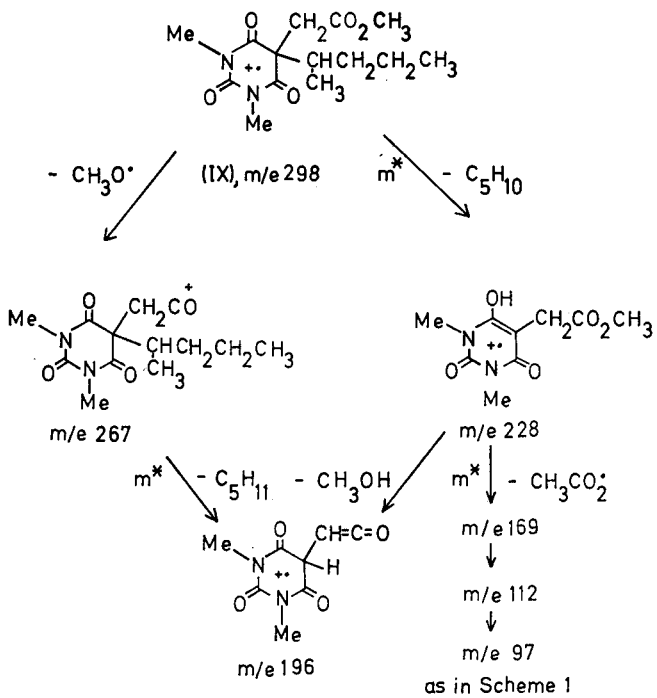
NN′-*Dimethyl*-3′-*hydroxypentobarbitone* (XII)
255 (2), 241 (2), 239 (2), 226 (26), 225 (6), 224 (30), 209 (3), 197 (2), 186 (7), 185 (44), 184 (89), 183 (18), 171 (5), 170 (11), 169 (100), 168 (2), 166 (3), 157 (4), 156 (6), 155 (4), 154 (2), 128 (7), 126 (11), 112 (21), 111 (7), 97 (18), 83 (9), 71 (10), 70 (19), 69 (85),67 (10), 59 (7), 58 (27), 57 (7), 56 (9), 55 (29), 53 (9), 45 (46), 44 (6), 43 (23), 42 (21), 41 (48), 40 (7), 39 (20), 30 (11), 29 (19).

The ester (IX), derived from quinalbarbitone by oxidation and subsequent methylation with diazomethane, undergoes fragmentations typical of the barbiturates and of esters (Scheme 2). Again the McLafferty rearrangement is in evidence in the typical elimination of the 1-methylbutyl side chain as an olefin from the molecular ion. The ester side chain in the resulting ion then loses a molecule of methanol.

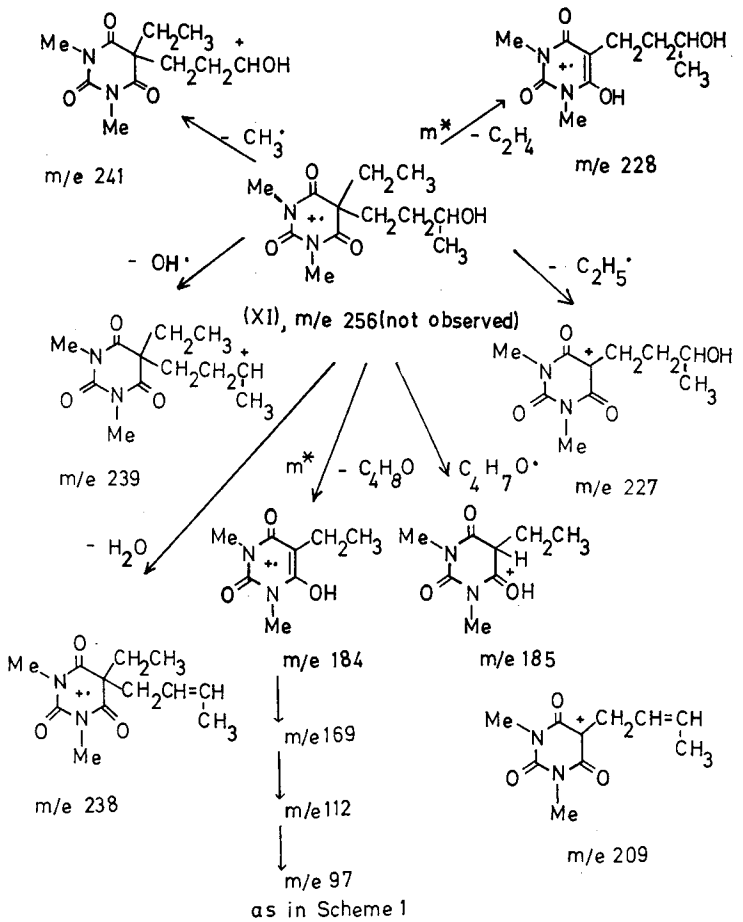
The compounds (XI) and (XII), with hydroxylated side-chains, behave in a similar manner to each other. Thus the side-chains fragment by loss of the terminal methyl; by loss of $CH_3CHOH$; by loss of water and by complete elimination via the McLafferty rearrangement. This latter gives an intense peak in both spectra, and elimination of a methyl radical from this ion gives rise to the base peak. The breakdown of *NN′*-dimethyl-3′-hydroxybutobarbitone (XI) is shown in Scheme 3.




*Scheme 1*




*Scheme 2*




*Scheme 3*

In conclusion, it is evident that mass spectrometry, combined with gas-liquid chromatography, offers a very powerful method of determining the structures of barbiturate metabolites present in quite complex mixtures, since the side-chains fragment by processes which enable their structures to be deduced.

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