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Keyphrases

Parabens, methyl, propyl-sucrose esters—interaction
Solubility method—parabens-sucrose esters interaction
Microbiological analysis—parabens-sucrose esters
UV spectrophotometry—analysis

Identification of Medicinal Barbiturates by Means of Mass Spectrometry

By R. T. COUTTS and R. A. LOCOCK

The mass spectra of the eight barbiturates most commonly prescribed in North America (amobarbital, barbital, butabarbital, mephobarbital, pentobarbital, phenobarbital, secobarbital, and thiopental) and, for comparison purposes, the spectra of butethal (butobarbitone B.P.) and itobarbital were recorded. The weakly acidic material present in capsules or tablets of four different barbiturate-containing products was extracted into ether and the mass spectrum of each extract was recorded. Evidence is presented that it is possible to identify positively individual barbiturates, and mixtures of barbiturates in pharmaceutical dosage forms, by means of mass spectrometry. The mass spectrum of phenacetin (present in one of the capsules studied) is discussed.

THE MASS SPECTRA of various barbiturates of medicinal importance (1) and other barbiturates (2) have been recorded and interpreted. The former study has revealed that under electron impact, barbiturate molecules undergo fragmentations in characteristic manner and the formation of the major ions in their mass spectra has been explained. The data as they are presented, however, do not permit easy differentiation between closely related structures. A positive identification of a particular barbiturate, such as would be desirable in toxicology studies, would not be possible using the reported (1) data.

The present study, therefore, was undertaken to determine whether it was possible to identify positively individual barbiturates, and to see whether mixtures of barbiturates in pharmaceutical dosage forms could be determined qualitatively, using mass spectrometry.

EXPERIMENTAL

Materials—Some of the barbiturates used in this study were purchased from the sources indicated: May and Baker (Canada) Ltd., Montreal (amobarbital, butethal, butabarbital, pentobarbital, and secobarbital); The British Drug Houses Ltd., London (barbital); Merck and Co., Ltd., Montreal (phenobarbital). The remainder were gifts from various drug houses.[1]

Isolation Procedure—*Capsules*—A portion (5 mg.) of the capsule contents was dissolved or suspended in water (2 ml.), concentrated hydrochloric acid (0.1 ml.) was added, and the whole extracted with ether (2 × 5 ml.). The combined ether extracts

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[1] Abbott Laboratories Ltd., Montreal (thiopental); C. E. Frosst and Co., Montreal (Twinbarb capsules); Eli Lilly and Co. (Canada) Ltd., Toronto (Tuinal capsules); A. H. Robins Company of Canada, Ltd., Montreal (Phenaphen capsules); Sandoz Pharmaceuticals, Dorval, Quebec (itobarbital and Plexonal tablets); Winthrop Laboratories, Aurora, Ontario (mephobarbital).

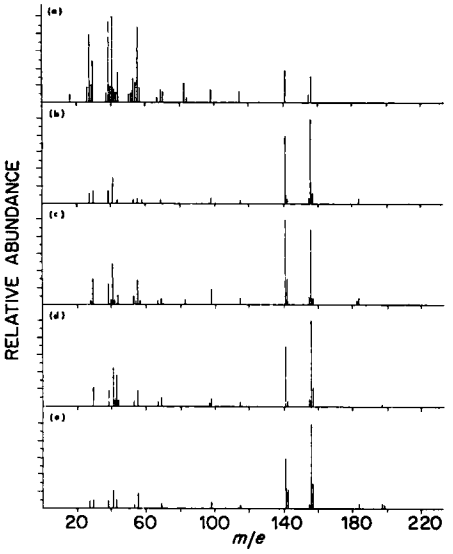


Fig. 1—Mass spectra of (a) barbital; (b) butabarbital; (c) butethal; (d) pentobarbital; and (e) amobarbital.

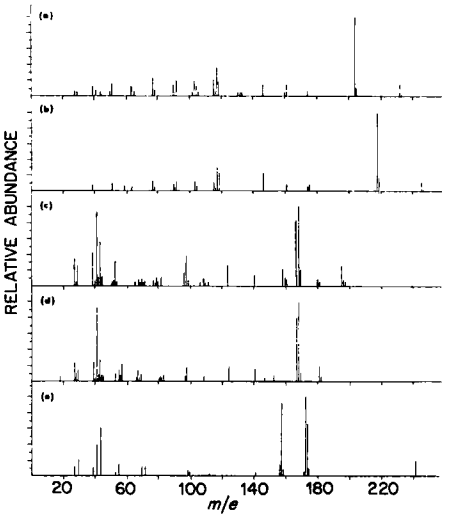


Fig. 2—Mass spectra of (a) phenobarbital; (b) mephobarbital; (c) secobarbital; (d) itobarbital; and (e) thiopental.

was extracted with sodium bicarbonate solution (2 × 2 ml., discarded), washed with water (2 ml.), and dried (Na_2SO_4). The ether solution so obtained was used for mass spectrum determination.

Tablets—A portion (10 mg.) of the powdered tablet was treated in the manner described for capsules.

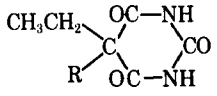
Mass Spectra—The mass spectra of the solids and ether extracts were determined using an A.E.I. M.S.9 instrument equipped with a heated inlet system operating at 150–170°. The electron beam energy was 70 ev. Samples were introduced by direct probe. Line spectra were drawn from the mass spectra and in each instance the most abundant ion was set at 100. Lines of relative abundance less than 2% were ignored in all figures except Fig. 3.

RESULTS AND DISCUSSION

Many deliberate and accidental deaths can be attributed to barbiturate poisoning. Summaries of cases of poisoning have been published (3, 4) and these have revealed that the vast majority of deaths are the result of ingestion of one or more of a relatively small number of barbiturates; amobarbital, bartital, butethal (butobarbitone), pentobarbital, phenobarbital, and secobarbital (quinalbarbitone) are the ones most commonly used. The mass spectra of these six barbiturates were recorded. In addition, the spectra of three other barbiturates, which are present in a significant number of preparations available in North America (5) were run. For comparison purposes, the spectrum of itobarbital was also recorded. Line diagrams of all 10 spectra were drawn (Figs. 1 and 2). These spectra are discussed conveniently under four groups.

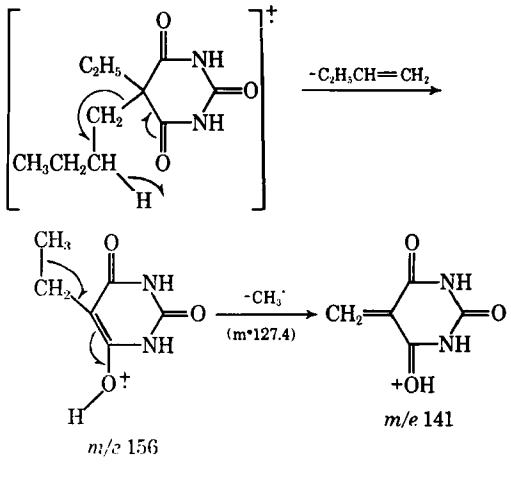
The first group comprises compounds of General Structure I. None of the compounds in this group (barbital, butabarbital, butethal, pentobarbital, and amobarbital) gave a parent ion, an observation which is contrary to what was reported previously (1). The mass spectrum of each of these five compounds, however, showed a very weak M+1 peak (rel. abund. 0.2–1.7%). Its presence together with the virtual absence of an M peak was at first confusing, but this observation can be explained in terms of the abstraction of a proton from the neutral barbiturate molecule by the molecular ion, to give a protonated molecular ion which is more stable than the molecular ion (6).

All of the compounds of General Structure I are



I, R = alkanyl

barbiturate	R
barbital	CH_3CH_2—
butabarbital	$CH_3CH_2CH(CH_3)$—
butethal	$CH_3CH_2CH_2CH_2$—
pentobarbital	$CH_3CH_2CH_2CH(CH_3)$—
amobarbital	$CH_3CH(CH_3)CH_2CH_2$—



Scheme I

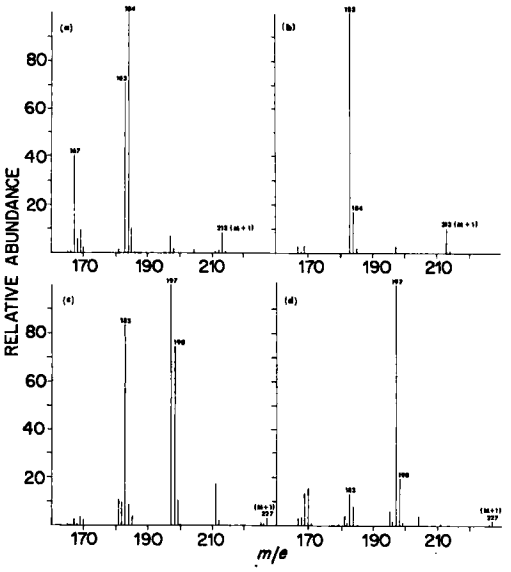
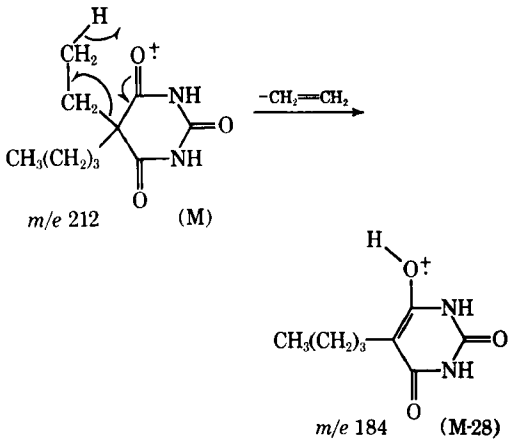


Fig. 3—Portions of mass spectra of (a) butethal; (b) butabarbital; (c) amobarbital; and (d) pentobarbital in the molecular ion region. The relative abundance of the strongest ion within the region is considered as 100.

known (1) to fragment in the manner illustrated in Scheme I for butethal, and give rise to strong ions of *m/e* 156 and *m/e* 141. The direct *m/e* 156→*m/e* 141 fragmentation is supported by the presence of a particularly strong metastable ion (m*) of *m/e* 127.4. Barbital is readily distinguished from the other four structures. It gave peaks of only medium intensity at *m/e* 156 and *m/e* 141 as well as the metastable ion at 127.4; abundant ions were located at *m/e* 55, 41, 39, 29, and 27 (Fig. 1*a*). In contrast, the mass spectra of butabarbital, butethal, pentobarbital, and amobarbital (Figs. 1*b*–1*e*) are extremely similar. Under electron impact, all readily lose the alkyl side chain. The resultant abundant ion, *m/e* 156, fragments in virtually identical fashion in all instances. Thus, to identify these compounds, reference must be made to that part of the spectrum in which masses greater than 156 are recorded. Appropriate line diagrams were drawn (Fig. 3).

The presence of the peak at *m/e* 197 in the spectra of amobarbital and pentobarbital readily distinguished these two barbiturates from butethal and butabarbital, in which this ion is absent. Butethal and butabarbital are isomeric, but nevertheless, it is possible to distinguish the one from the other by means of its mass spectrum. The former has an unbranched butyl substituent at C_5. This increases the amount to which the fragmentation depicted in Scheme II occurs and thus increases the relative abundance of the M-28 ion. A weak metastable ion, *m/e* 151.7, supports the subsequent loss of an OH radical from the M-28 ion, which results in the formation of an ion, *m/e* 167 (*i.e.*, M-45 ion), of significant abundance. In contrast, the 1′-methyl substituent of the C_5-isobutyl side-chain of butabarbital apparently minimizes an M→M-28 fragmentation in this compound. The result is that the M-28 ion of butabarbital is of very low abundance, the M-45 ion is virtually absent, and a metastable ion at *m/e* 151.7 is not observed.



Scheme II

The other two barbiturates, amobarbital and pentobarbital, are also isomeric compounds, and they can be differentiated in a similar way. The former does not have a 1′-methyl substituent in the C_5H_{11} side chain. The abundance of the M-28 (*m/e* 198) and the M-43 (*m/e* 183) ions from this compound are significant. The latter fragment is reputed (1) to be the M-HNCO ion. In addition, a weak metastable ion, *m/e* 165.5, in the spectrum of amobarbital supports a direct fragmentation of M-28→M-45 (loss of ·OH). The presence of the 1′-methyl substituent in the C_5H_{11} side chain of pentobarbital again minimizes an M→M-28 transition and thus the *m/e* 198 ion from this compound is of very low abundance. Also, no metastable ion is observed at *m/e* 165.5 in this spectrum.

Phenobarbital and mephobarbital (II*a* and II*b*) are readily identified by means of their mass spectra (Figs. 2*a* and 2*b*). Both spectra show a parent ion, of low relative abundance, of *m/e* 232 and 246, respectively, and the base peak in both spectra is the result of the loss of the ethyl side chain as an ethylene molecule (*cf.* Scheme II). The presence of a strong metastable ion, *m/e* 179.2, in the spectrum of phenobarbital and the equivalent ion, *m/e* 193.2, in the mephobarbital spectrum support a direct M→M-28 cleavage of both molecules. The presence of these metastable ions is of value in identifying these two barbiturates.

The third group of compounds investigated possess the General Structure III. Parent peaks (*m/e* 238 and 224, respectively) were absent from the mass spectra of secobarbital and itobarbital; M+1 ions (*m/e* 239 and 225, respectively) were present in less than 1% abundance. The most diagnostic features of the mass spectra of secobarbital (Fig. 2*c*) and itobarbital (Fig. 2*d*), as well as butalbital (1), are the presence of two very abundant ions of *m/e* 168 and 167, both of which arise by the loss of the saturated C_5-substituent. Thus, the ion of *m/e* 168 in the spectrum of secobarbital is formed by loss of $CH_3CH_2CH_2CH{=}CH_2$ from the molecular ion; loss of the radical $CH_3CH_2CH_2\dot{C}HCH_3$ accounts for the formation of the ion, *m/e* 167. Itobarbital and butalbital fragment in a similar way. The result is that the mass spectra of secobarbital, itobarbital, and butalbital are virtually identical. Minor, but significant differences are observed above *m/e* 168,

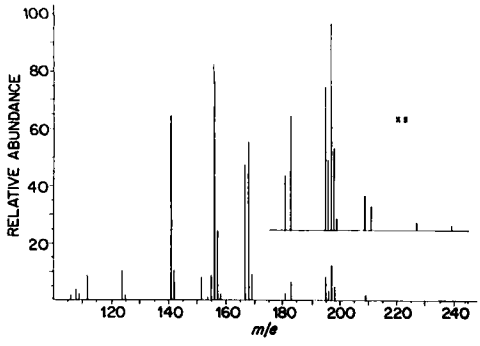


Fig. 4—Mass spectrum of an extract from a capsule which contained amobarbital and secobarbital.

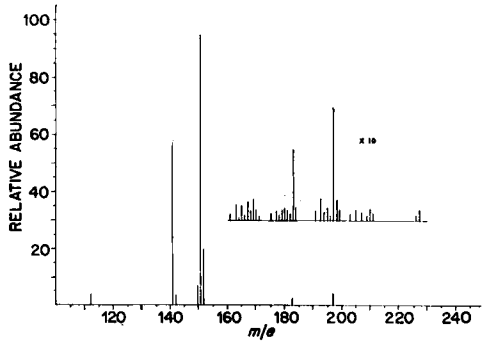
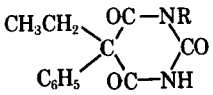


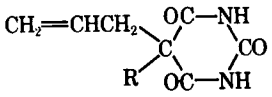
Fig. 5—Mass spectrum of an extract from a capsule which contained pentobarbital and butabarbital.

and in this way, it is possible to differentiate secobarbital from itobarbital, even though molecular ions are absent from both spectra. The former molecule loses a propyl radical from the alkanyl side chain of the molecular ion, and gives an ion of significant abundance (26%) of *m/e* 195 (*m/e* calcd.



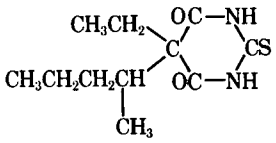
II

	R
(*a*) phenobarbital	H
(*b*) mephobarbital	CH_3



III

	R
(*a*) secobarbital	$CH_3CH_2CH_2CH(CH_3)$—
(*b*) itobarbital	$CH_3CH(CH_3)CH_2$—
(*c*) butalbital	$CH_3CH_2CH(CH_3)$—



IV

for $C_9H_{11}N_2O_3$: 195.0770; measured: 195.0764). Similarly, the loss of an ethyl radical gives an ion of low abundance (3%) of *m/e* 209. The former ion is absent from the mass spectrum of itobarbital.

The structure of thiopental (IV) is related to those compounds of General Structure I and fragments in a similar manner (Fig. 2*e*). The presence of the sulfur atom stabilizes the molecular ion, however, and an M peak (rel. abund. 20%), *m/e* 242, is observed. The other major diagnostic features in the mass spectrum of this molecule are abundant ions of *m/e* 172 and 157 and a strong metastable ion of *m/e* 143.3. These ions correspond to the ions of *m/e* 156, 141, and 127.4, respectively, in compounds of Structure I, and are formed in the same manner (*cf.* Scheme I).

To determine whether mass spectrometry could be used to identify barbiturates in pharmaceutical dosage forms, four commercially available products, each of which contained at least one of the barbiturates already discussed in this study in combination with other medicaments, were selected. Three of the products were capsules and one was a sugar-coated tablet. Ether extracts of the weakly acidic materials (and neutral materials, if present) contained in 5 mg. of each capsule or 10 mg. of the powdered tablet were made, and the mass spectra of all four ether extracts were recorded. As ions of small mass are of limited value in fragmentation studies, a line diagram of the ions of *m/e* 100 (arbitrarily chosen) and greater mass was drawn from each spectrum. The four diagrams so obtained (Figs. 4–7) differed appreciably from each other.

In the spectrum (Fig. 4) of the extract from a capsule which contained sodium amobarbital and sodium secobarbital,[2] the abundant ions of *m/e* 156 and 141 together with the strong metastable ion *m/e* of 127.4 confirmed the presence of a barbiturate of General Structure I; the two abundant ions of *m/e* 168 and 167 indicated that a barbiturate of Structure III was also present. Consideration of the *m/e* 180–240 region permitted more positive identification. The very weak peaks of *m/e* 239 and 209 and the weak peak of *m/e* 195 are all found in the spectrum of secobarbital and correspond to the M+1, M-29, and M-43 ions, whose formation has been discussed earlier. The presence of a very weak peak of *m/e* 227 and weak peaks of *m/e* 198, 197, and 183 suggest amobarbital, rather than its isomer (pentobarbital).

The simple mass spectrum (Fig. 5) of the extract from a capsule containing sodium pentobarbital and butabarbital,[3] with its abundant ions of *m/e* 156 and 141 (and a very strong metastable ion of *m/e* 127.4) clearly indicates that one or more compounds of General Structure I are present in this preparation. The absence of M peaks and the very low abundance of M+1 peaks makes a positive identification of pentobarbital and butabarbital impossible. However, when reference is made to the *m/e* 160–230 portion of the spectrum of the extract and this is compared with the information included in Fig. 3, then some compounds of Structure I may be eliminated as possible components of the preparation. The intensities of the ions of *m/e* 184 and 198 (Fig. 5) would tend to eliminate butethal and amobarbital from consideration, for example.

[2] Tuinal capsules (Eli Lilly and Co. Ltd.).
[3] Twinbarb capsules (C. E. Frosst and Co.).

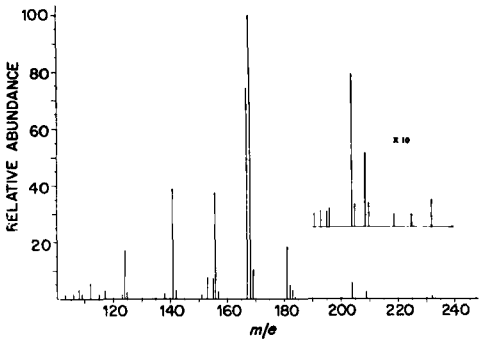


Fig. 6—Mass spectrum of an extract from a tablet which contained barbital, phenobarbital, butalbital, and other medicinal agents.

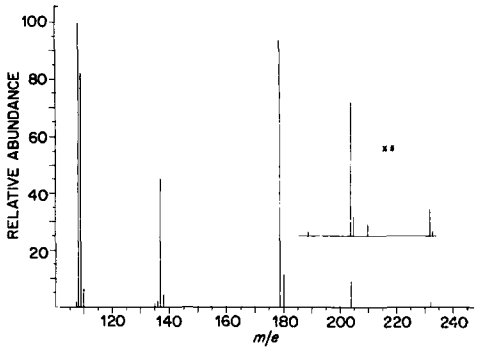
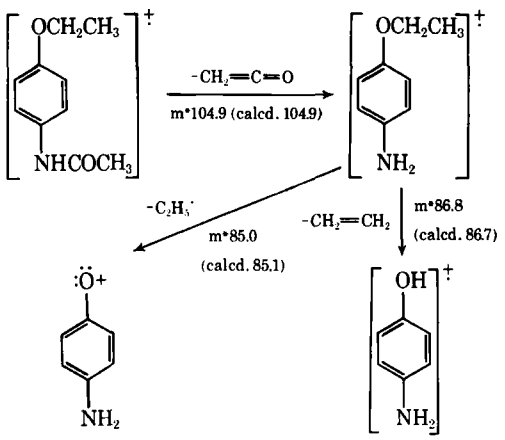


Fig. 7—Mass spectrum of an extract from a capsule which contained phenobarbital, phenacetin, and other medicinal agents.

The tablet studied contained sodium barbital, sodium phenobarbital, sodium butalbital, dihydroergotamine methanesulfonate, and scopolamine hydrochloride.[4] The mass spectrum (Fig. 6) of the ether extract of this tablet is quite diagnostic and indicates that a mixture of three barbiturates are present in the extract. A barbiturate of General Structure I is indicated by the presence of abundant ions of *m/e* 156 and 141 and the strong metastable ion of *m/e* 127.4. A positive identification of this barbiturate is not possible, although a comparison of



Scheme III

the region *m/e* 182–232 with the data in Fig. 3 eliminates butethal, amobarbital, and pentobarbital as possibilities. The presence of itobarbital in the mixture is indicated by the abundant ions of *m/e* 168 and 167 and by the absence of a significant ion of *m/e* 195 (which is present in the spectra of secobarbital and butalbital). The weak peaks of *m/e* 232 and 204 confirm the presence of phenobarbital in the mixture.

The dominant features in the spectrum (Fig. 7) of the ether-soluble neutral and acidic material present in the fourth commercial product[5] studied (which contained phenacetin, acetylsalicylic acid, phenobarbital, and hyoscyamine sulfate) are abundant ions of *m/e* 179, 137, 109, and 108, all of which arise from electron bombardment of the phenacetin molecule in the manner shown (Scheme III). The presence of strong metastable ions (m*) of appropriate masses confirm these fragmentation pathways.

In addition to these abundant ions, significant ions of *m/e* 232 and 204 were observed. Their presence together with a metastable ion of *m/e* 179.2, which was somewhat obscured by the strong parent peak of phenacetin, indicated the presence of phenobarbital in the extract. No attempt was made to remove the phenacetin before recording the mass spectrum. Had this been done, the spectrum would have been characteristic of many preparations containing phenobarbital as the sole barbiturate. The retention of phenacetin in the ether extract makes the mass spectrum much more diagnostic.

CONCLUSION

It has been demonstrated that it is possible to identify individual barbiturates by means of mass spectrometry. Barbiturates in pharmaceutical dosage forms can be identified also in this way. The results obtained in this study suggest that a library could be assembled of the mass spectra of extracts from pharmaceutical preparations containing barbiturates. The spectra so collected could be used as reference spectra in forensic studies, when only a very small portion of the pharmaceutical preparation is available.

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Keyphrases

Barbiturates—identification
Dosage forms, barbiturates—analysis
Mass spectroscopy—identity, structure
Electron impact—barbiturate fragmentation

[4] Plexonal tablets (Sandoz Pharmaceuticals).
[5] Phenaphen capsules (A. H. Robins Co. Ltd.).