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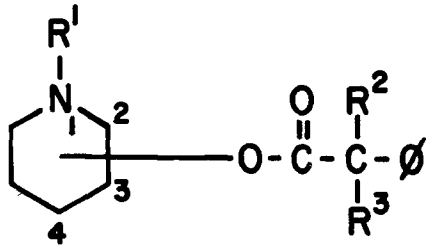
Mass Spectrometry as an Aid in the Detection and Identification of Piperidyl Benzilates and Related Glycolates

In 1958, Abood and co-workers [*1*] synthesized a series of compounds with the general structure indicated by Compound I (Fig. 1), using the piperidine ring of atropine and replacing the tropic acid side chain with substituted glycolic acids. In recent years, considerable attention has been given to this series of compounds because of their potent anticholinergic and central nervous system (CNS) activity [*2–6*]. In man, they exhibit powerful, psychotomimetic, and antidepressant symptoms, and, in doses of 1 to 25 mg, produce long-lasting hallucinogenic effects similar in action to lysergic acid diethylamide (LSD). These compounds are also quite toxic and in larger doses can lead to death through respiratory failure.



I

FIG. 1—*General structure of compounds studied.*

Referring to Fig. 1, maximum hallucinogenic activity is exhibited when R^1 is a small (methyl or ethyl) alkyl group and R^2 is a hydroxyl group. The magnitude of CNS activity is also determined by R^3, the potency decreasing in the order of cycloalkyl, phenyl, unsaturated alkyl, and alkyl. Of the cycloalkyl derivatives, potency and duration of

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CNS activity is greatest when R^3 = cyclopentyl [7]. In addition to the above, CNS activity is also strongly influenced by the position of substitution on the piperidyl ring. Substitution at the 4 position induces the greatest psychotomimetic activity, and this becomes progressively lower for the 3 and 2-positional isomers.

Because of their hallucinogenic properties, piperidyl glycolates and related compounds are of interest to the Drug Enforcement Administration (DEA). The compounds of current forensic concern among them are the benzilate esters JB-336 (IV) and JB-318 (V), which are listed under Schedule I classification by the DEA. These compounds have appeared in illicit trade since 1967 under such acronyms as "LBJ" and "TWA."

Information regarding the identification of piperidyl benzilates and related compounds is very scarce in the literature. Gunn [8] has reported that there is no published method for analysis, and the manual of the Bureau of Narcotics and Dangerous Drugs (now DEA) indicates no standard method for the detection or quantitation of benzilate esters [8,9]. Furthermore, in a survey of the more recent literature we have found no additional information regarding a definitive method for the analysis of these compounds.

Ultraviolet (UV) spectroscopy has been used with very limited success. Although the spectra are well defined, the extinction coefficients are too low for trace analysis. In addition, the UV spectra of JB-336 and JB-318 are identical with benactyzine, a powerful hallucinogen which contains the benzilate group and which is a component in several legitimate preparations [9]. Limited data on the analysis of glycolate esters by chromatographic techniques have been published [10,11]. The latter studies, however, were mainly concerned with the analysis of benactyzine which was present in the specific pharmaceutical mixtures investigated. Verweij et al [12] used thin-layer chromatography (TLC) to establish relative retention (R_f) values for a number of glycolic acid esters, including the piperidyl benzilates. Identification was established by comparison of their R_f values on alumina and silica gel in various adsorption systems.

In view of the needs for more specific and selective methods for the analysis of the wide variety of compounds of this type, we have examined in detail the mass spectral characteristics of a series of piperidyl benzilate esters and related compounds kindly made available to us by the DEA. This study was motivated, in part, by the desire to establish some simple criteria for the detection and elucidation of the structure of such compounds. The particular series investigated provided sufficient structural diversity and functional group variation to help understand some of the parameters governing the fragmentation of the individual compounds. Of particular significance is the recognition of positional and structural isomers of JB-336 (IV) and JB-318 (V), which may often be present in an illicit drug sample. The mass spectra of the compounds are presented in the following section. Fragmentations are discussed first in terms of the benzilate and piperidine moieties and then by a consideration of other special spectral features that are characteristic of the individual compounds investigated.

Experimental

Mass spectra were obtained with a Nuclide 12-90-G single-focusing magnetic mass spectrometer operating in the electron impact mode. The accelerating voltage was 4.5 kV, trap current 50 μA, and ionizing energy 70 eV. The ion source temperature was maintained at 225°C (437°F). Samples were introduced via the direct inlet probe. The probe tip temperature for sample vaporization into the ion source did not exceed 100°C (212°F) for most cases, and there was no evidence of any thermal decomposition. Gas chromatographic (GC) analysis gave no indication of major impurities in the specific drug samples examined. The GC used was a Pye 104 equipped with dual flame ionization detectors, and separation was accomplished with a 6-ft (1.8-m) by 4-mm inside diameter glass column packed with 3% SP-2250 on Supelcoport 80/100 mesh.

Results and Discussion

The compounds examined in this study, their structural formulas, and commercial code numbers are shown in Figs. 2–5. Figures 6–9 show the mass spectra of these drugs. Compounds II and III (Fig. 6) are positional isomers, while IV–VI (Fig. 7) are isomers or homologs, or both, representing a variation in both *N*-alkyl and ring substitution. Compounds VII–IX (Fig. 8) were selected to evaluate the effect of structural variation in the glycolate moiety on the mass spectral pattern, and finally compounds X–XII (Fig. 9) are representative of the variation of the piperidine nitrogen substitution on the mass spectral fragmentation pattern.

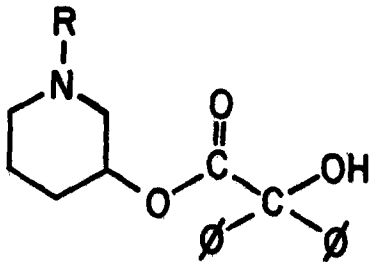

FIG. 2—*Compound II*: *R* = *H*; *3-piperidylbenzilate* (*JB-841*). *Compound IV*: *R* = CH_3; N-*methyl-3-piperidylbenzilate* (*JB-336*). *Compound V*: *R* = C_2H_5; N-*ethyl-3-piperidylbenzilate* (*JB-318*).

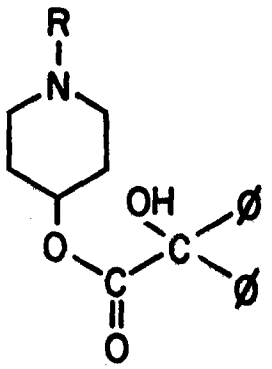

FIG. 3—*Compound III*: *R* = *H*; *4-piperidylbenzilate* (*JB-8015*). *Compound VI*: *R* = CH_3; N-*methyl-4-piperidylbenzilate* (*JB-8191*).

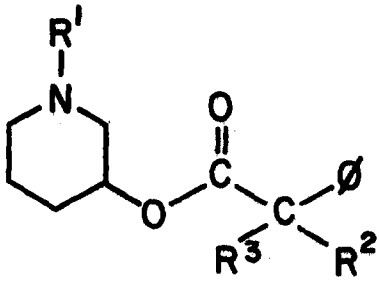

FIG. 4—*Compound VII*: R^1 = C_2H_5, R^2 = ϕ, R^3 = *H*; N-*ethyl-3-piperidyldiphenylacetate* (*JB-305*). *Compound VIII*: R^1 = C_2H_5, R^2 = *OH*, R^3 = *cyclopently*; N-*ethyl-3-piperidylphenylcyclopentyl glycolate* (*JB-478*). *Compound IX*:R^1 = CH_3, R^2 = *OH*, R^3 = *cyclohexyl*; N-*methyl-3-piperidylphenylcyclohexyl glycolate* (*JB-840*).

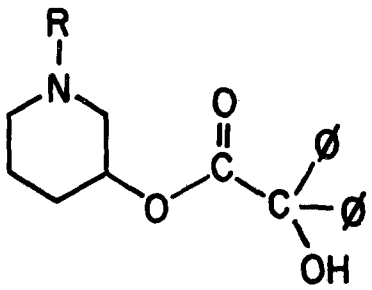

FIG. 5—*Compound X*: $R = CH_2{=}CH\text{-}CH_2$; N-*allyl-3-piperidylbenzilate* (*JF-18*). *Compound XI*: $R = \phi\text{-}CH{=}CH\text{-}CH_2$; N-*cinnamyl-3-piperidylbenzilate* (*JB-8008*). *Compound XII*: $R = (CH_3)_2\text{-}N\text{-}CH_2\text{-}CH_2$; N-(*dimethylaminoethyl*)-*3-piperidylbenzilate* (*JB-851*).

The mass spectra of all the compounds investigated exhibited easily recognizable molecular ion peaks of generally low relative intensity. Fragment ions are formed by retention of the charge with either the piperidyl or the glycolate moieties. The presence of the benzilate function can be ascertained by the characteristic series of ions at mass to charge ratios (*m/e*) 183, 165, 105, and 77. Preliminary screening for the benzilate system can be best accomplished by selective monitoring of the *m/e* 183 ion. This is a more logical choice than either *m/e* 105 or 77 since the latter ions (and more specifically, *m/e* 77) are typical of compounds containing an aromatic system. Moreover, the 183 ion is preferable to that at *m/e* 165 in view of the usually higher relative intensity of the former ion. Mechanisms for the formation of all four ions, as supported by metastable transitions and deuterium labeling of the hydroxylic hydrogen, are illustrated in Fig. 10. Loss of water from *m/e* 183 to produce *m/e* 165 requires a rather complex rearrangement process, which cannot easily be ascertained without additional isotopic labeling. The conjugated species *b* is, however, a logical structure for the resulting fragment ion.

Cleavage of the piperidyl-ester oxygen bond and retention of the charge with the piperidyl function invariably resulted in the formation of highly abundant ions whose mode of further fragmentation was governed by the position of benzilate substitution, as discussed below for the individual types of compounds.

In examining the spectrum of Compound II, it is noted that cleavage of the piperidyl-ester oxygen bond results in ion *e* formed by loss of a hydrogen, presumably from C-2, to produce a conjugated species of *m/e* 83, as shown in Fig. 11. It should be noted that although an ion of *m/e* 84 is also formed in the spectrum of Compound II, the latter ion occurs to a much lesser extent than *m/e* 83 because of the tendency to eliminate a hydrogen and form the conjugated system *e*. Of major significance is the fact that in the spectrum of the positional isomer III, cleavage of the same bond is followed by loss of two hydrogens to produce the doublet at *m/e* 84–*m/e* 82 (ions *f* and *g*, Fig. 12). The latter mechanism provides the basis for differentiation of positional isomers of this type as illustrated further in the examples presented below.

The spectra of *N*-methyl or *N*-ethyl piperidylbenzilates (IV and V, Fig. 7) are consistent with that of Compound II. Retention of the charge with the piperidine ring results in the appropriate mass shifts to *m/e* 97 and 111 (homologs of *e*). Furthermore, the spectrum of the 4-substituted isomer (VI) exhibits an intense peak at *m/e* 98 and, consistent with its homolog III, further eliminates H_2 (or $2H\cdot$) to give the ion at *m/e* 96. In the spectra of the *N*-ethyl derivatives (V, VII, and VIII), notable is the loss of a methyl group resulting in the formation of $[M\text{-}15]^+$ ions. In the spectra of the same compounds the *m/e* 111 ion further eliminates a methyl group to give the conjugated ion *h* (*m/e* 96, Fig. 13).

As expected, the series of ions (*m/e* 183, 165, 105, and 77) characteristic of the benzilate

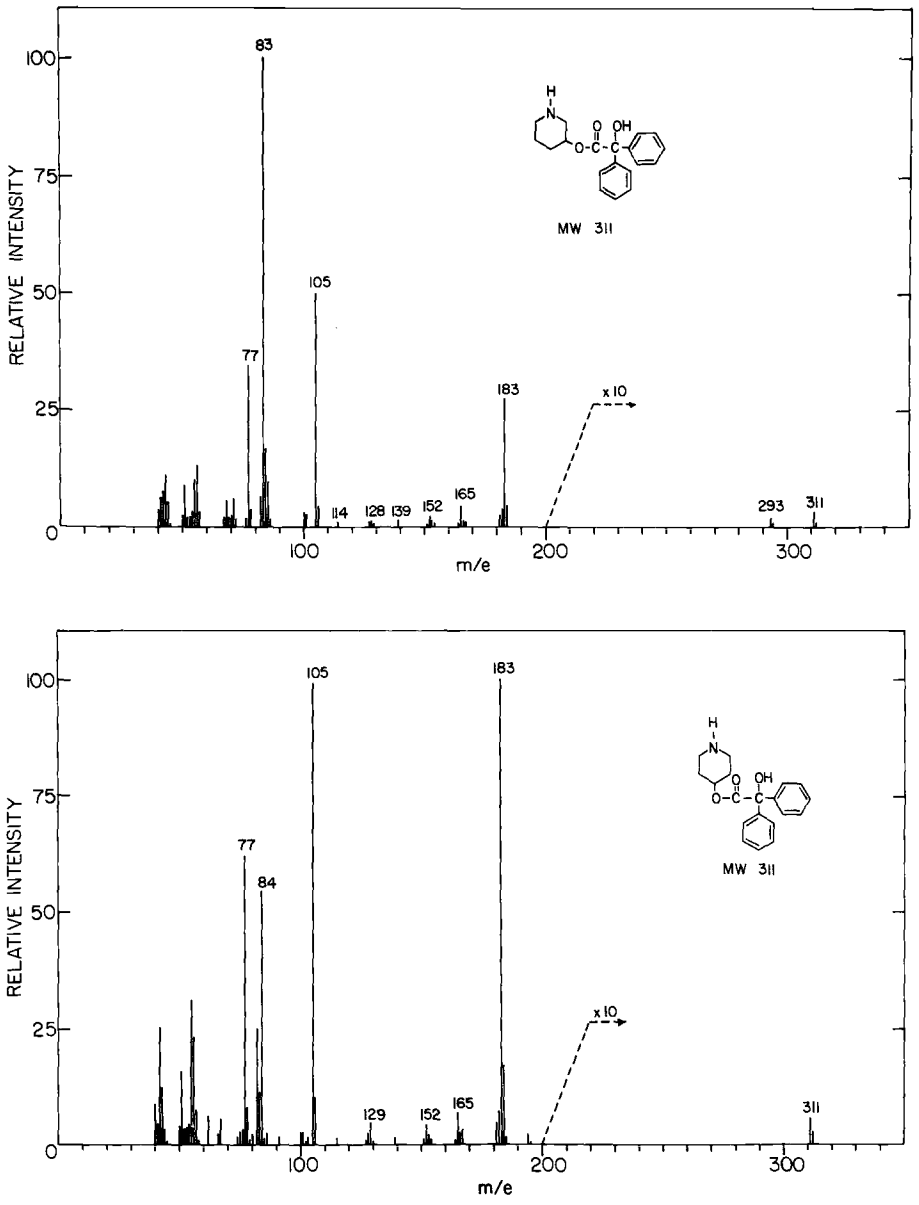

FIG. 6—*Mass spectra of 3-piperidylbenzilate* (*II,* top) *and 4-piperidylbenzilate* (*III,* bottom).

group is not present in the spectra of compounds VII–IX. However, ions typical of the piperidine ring, carrying a C-3 substituent (*m/e* 97 and *m/e* 111) are distinctly present. In a manner analogous to the formation of ion *a* (*m/e* 183), cleavage of the C-C bond β to the phenyl group gives rise to the peaks at *m/e* 167, 175, and 189 in the spectra of VII, VIII, and IX, respectively. In the case of the diphenylacetate derivative (VII), the ion of *m/e* 167 readily eliminates H_2 to give ion *b'* (*m/e* 165), presumably of structure similar to *b*, as shown in Fig. 14. The presence of cyclopentyl and cyclohexyl groups in the glycolates VIII and IX is reflected in the respective losses of those radicals to produce

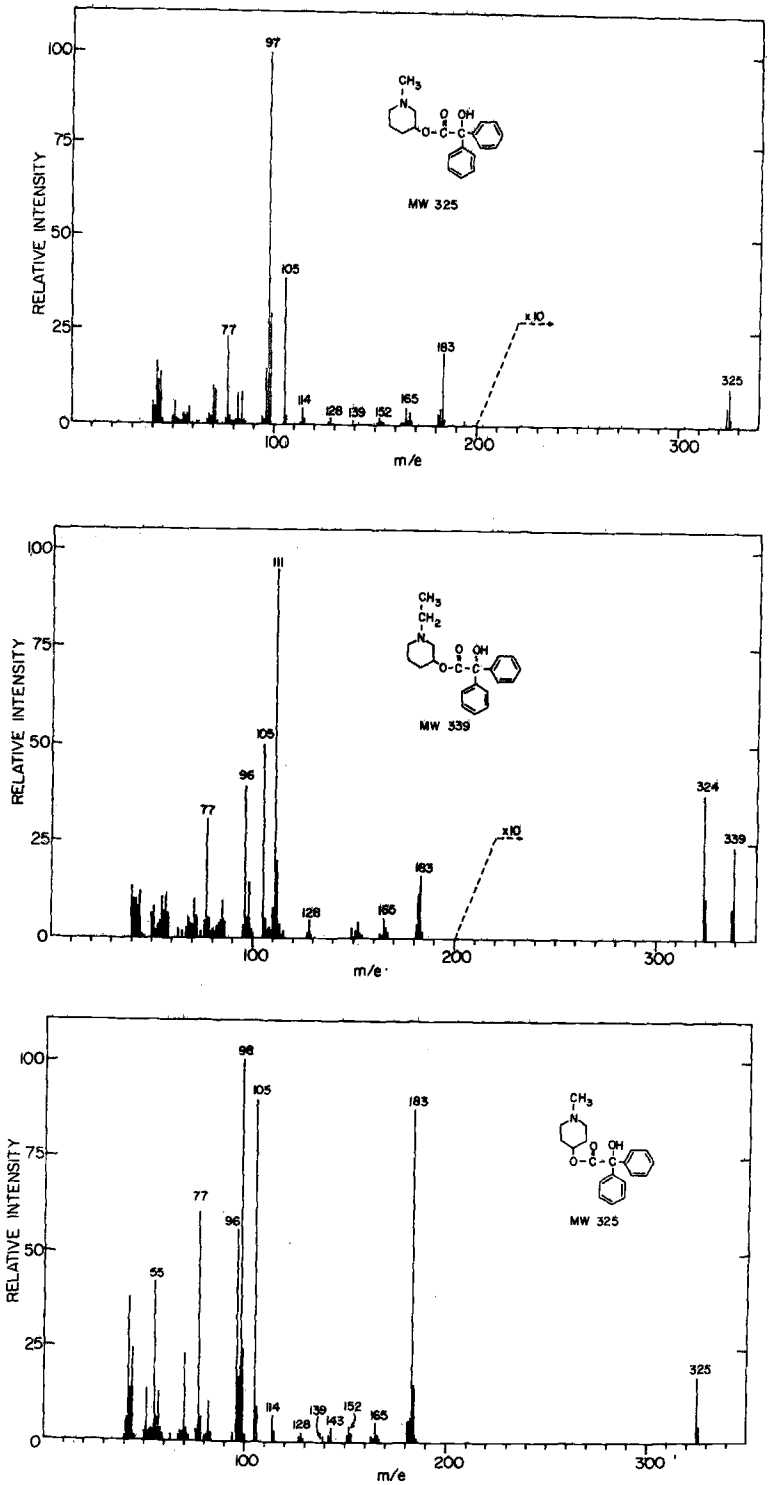

FIG. 7—*Mass spectra of* N-*methyl-3-piperidylbenzilate* (*IV,* top), N-*ethyl-3-piperidylbenzilate* (*V,* center) *and* N-*methyl-4-piperidylbenzilate* (*VI,* bottom).

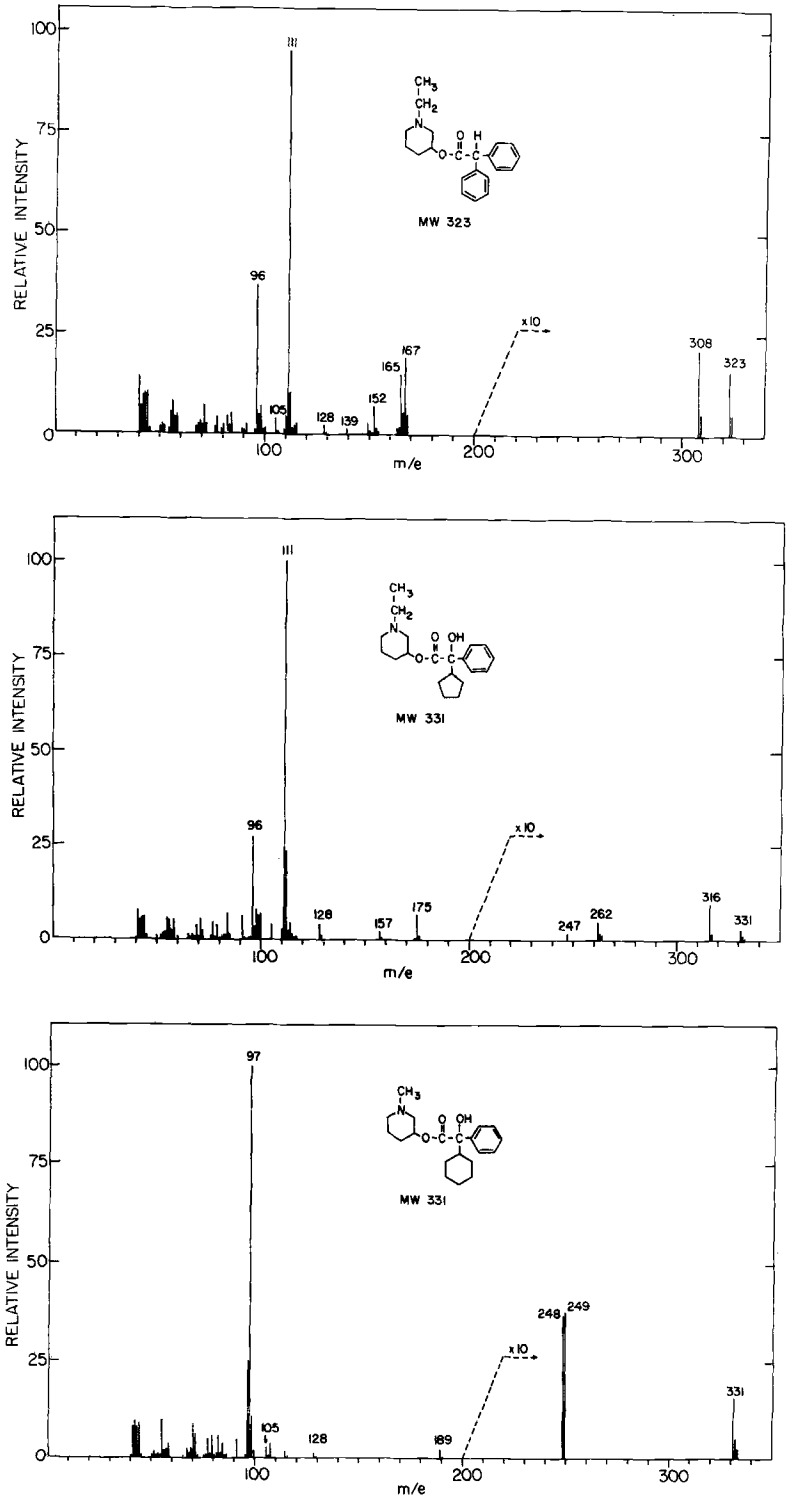

FIG. 8—*Mass spectra of* N-*ethyl-3-piperidyldiphenylacetate* (*VII,* top), N-*ethyl-3-piperidylphenylcyclopentyl glycolate* (*VIII,* center) *and* N-*methyl-3-piperidylphenylcyclohexyl glycolate* (*IX,* bottom).

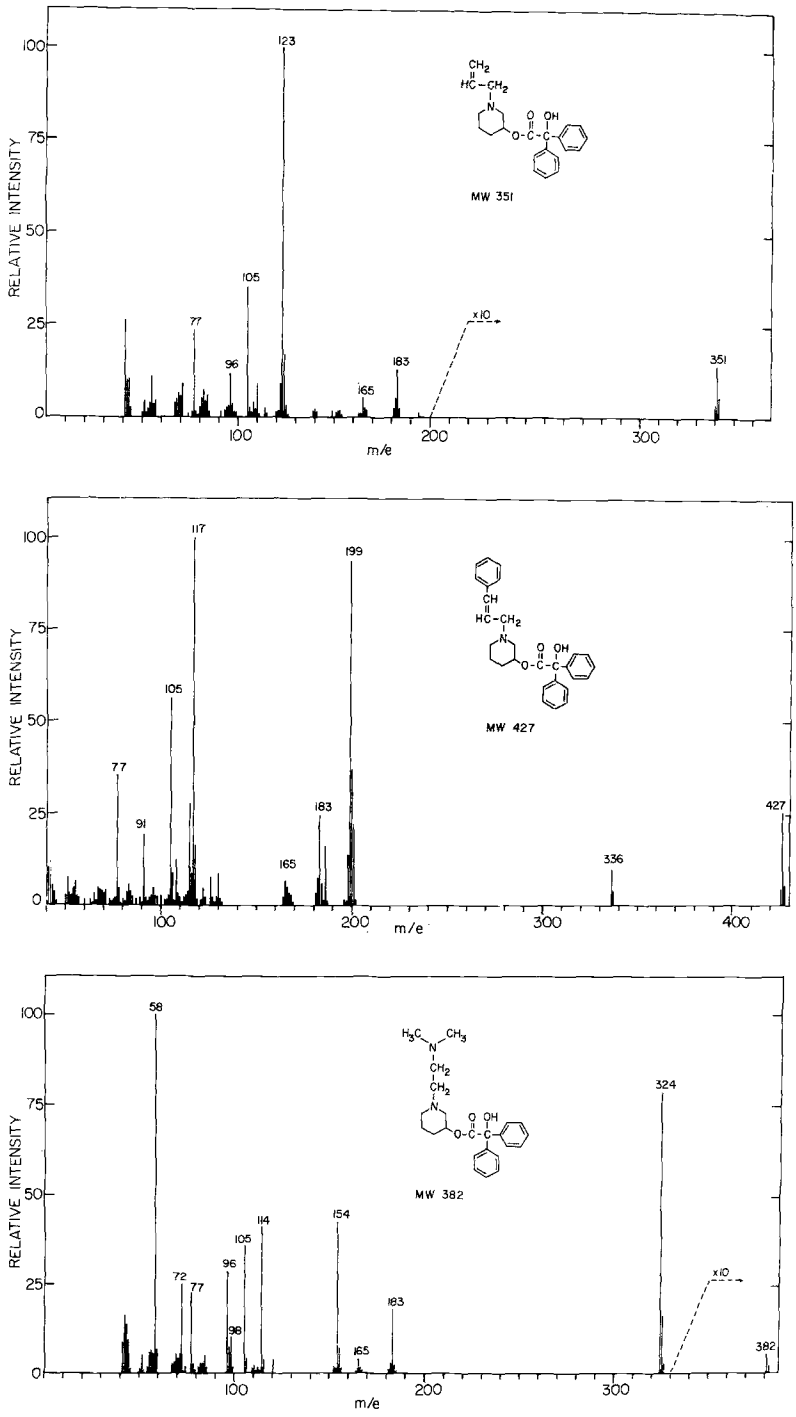

FIG. 9—*Mass spectra of* N-*allyl-3-piperidylbenzilate* (*X,* top), N-*cinnamyl-3-piperidylbenzilate* (*XI,* center) *and* N-(*dimethylaminoethyl*)-*3-piperidylbenzilate* (*XII,* bottom).

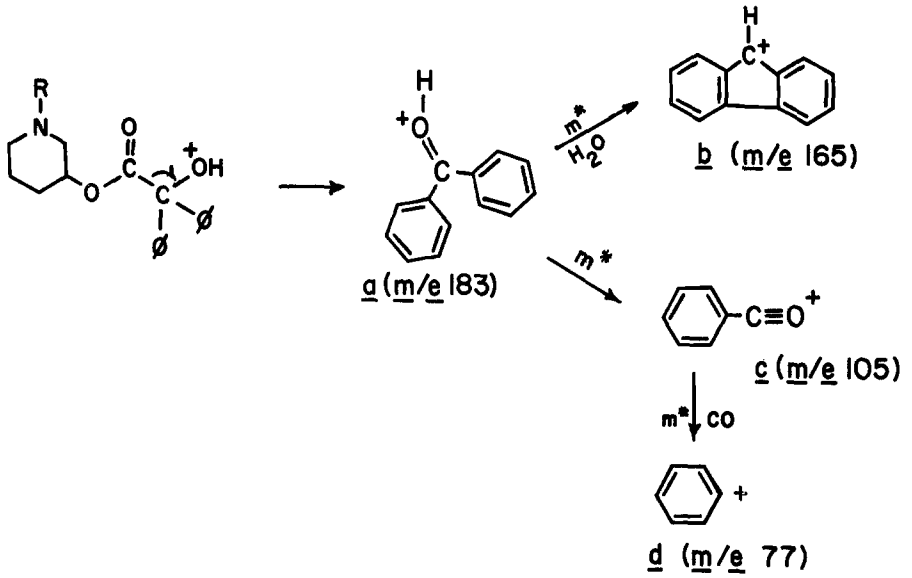

FIG. 10—*Mechanisms for the formation of four ions, as supported by metastable transitions and deuterium labeling of the hydroxylic hydrogen.*

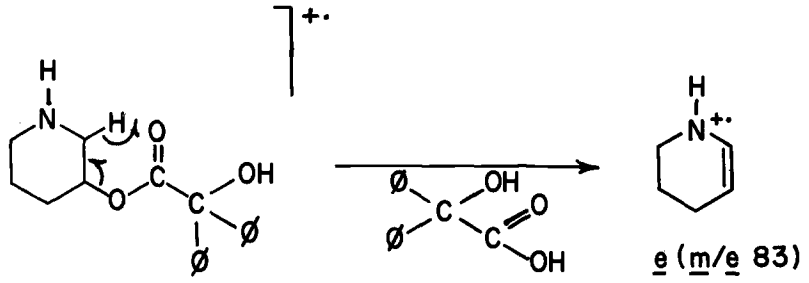

FIG. 11—*The cleavage of the piperidyl-ester oxygen bond of Compound II results in ion* e *formed by loss of a hydrogen, presumably from C-2, to produce a conjugated species of* m/e *83.*

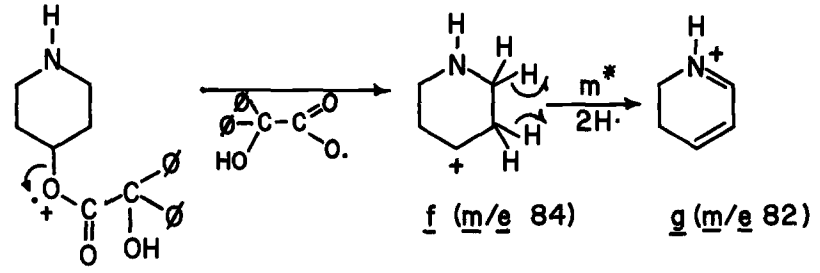

FIG. 12—*In the spectrum of the positional isomer III, cleavage of the same bond is followed by loss of two hydrogens to produce the doublet at* m/e *84*—m/e *82* (*ions* f *and* g).

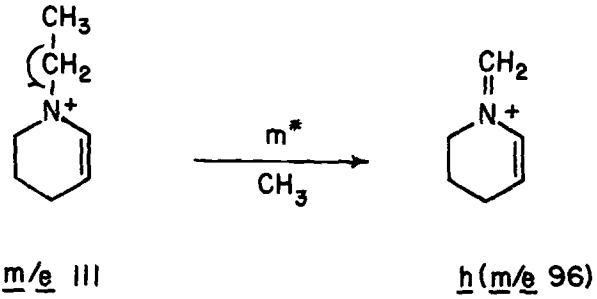

FIG. 13—*In the spectra of the* N-*ethyl derivatives (V, VII, and VIII), the* m/e *111 ion eliminates a methyl group to give the conjugated ion* h (m/e *96*).

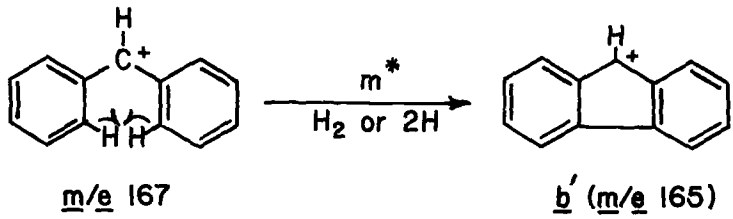

FIG. 14—*In the diphenylacetate derivative (VII), the ion of* m/e *167 readily eliminates* H_2 *to give ion* b' (m/e *165*), *presumably of structure similar to* b.

the ions of *m/e* 262 and 248. Elimination of cyclohexene, presumably via a McLafferty rearrangement [*13*], is responsible for the peak at *m/e* 249 in the spectrum of Compound IX (Fig. 15).

In the spectra of the benzilates containing a variation in *N*-piperidyl substitution (X, XI, and XII; Fig. 9), the same overall consistency of fragmentation was observed. This is reflected in the series of abundant ions at *m/e* 77, 105, and 183 characteristic of the benzilate group in all the spectra of Fig. 9 and the piperidyl ring-containing ions at *m/e* 96 and 123 in Compound X, *m/e* 199 in Compound XI, and *m/e* 96 and 154 in Compound XII. In addition, prominent are the ions characteristic of the *N*-piperidyl substituent as exemplified by the peak at *m/e* 117 (C_6H_5-CH=CH-CH_2^+) in the spectrum of the *N*-cinnamyl derivative. The latter ion fragments further to *m/e* 91 (C_6H_5-CH_2^+), a process characteristic of cinnamyl alcohols and related compounds [*14*]. The presence of the

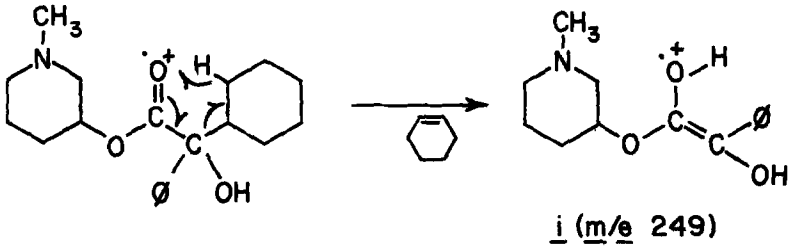

FIG. 15—*Elimination of cyclohexene is responsible for the peak at* m/e *249 in the spectrum of Compound IX.*

N,N-dimethylaminoethyl group on the piperidine nitrogen in Compound XII is reflected in the ions at *m/e* 324 $(M\text{-}58)^+$, *m/e* 58 $[(CH_3)_2{}^+N{=}CH_2]$, and *m/e* 72 $[(CH_3)_2N\text{-}CH_2\text{-}CH_2{}^+]$, which is consistent with the fragmentation of structurally related *N,N*-dimethylphenylethylamines and tryptamines [*15*]. Finally, the ion at *m/e* 114 in the spectrum of Compound XI corresponds to the formation of ion *k* as suggested in Fig. 16.

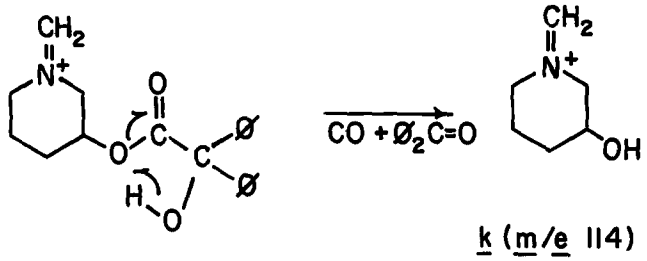

FIG. 16—*Suggested formation of ion* k.

Summary

On the basis of the data presented above the following conclusions may be drawn.

1. The molecular ion peaks of most of the compounds examined are relatively weak but usually easily discernible to permit molecular weight determination.
2. The mass spectra of benzilate esters exhibit a relatively intense peak at *m/e* 183, and monitoring of this ion can serve as a means for preliminary screening for the presence of this type of a system.
3. Related esters exhibit a similar type of fragmentation resulting in a fragment ion analogous to *m/e* 183 but shifted by the appropriate number of mass units according to the substituents present.
4. Cleavage of the piperidine ring-ester oxygen bond in 3 and 4-substituted isomers is followed by selective losses of hydrogen radicals to produce ions of type *e*, *f*, and *g* as indicated above. It is significant that in a related piperidine ring system (methylphenidate) substituted in the 2 position, the same type of cleavage results in no further hydrogen losses [*16*] because of charge stabilization from the ring nitrogen (ion *j*, Fig. 17) [*17*]. In other words, the tendency to form a conjugated ion following initial bond cleavage can serve as a means for identifying the position of substitution on the ring and for distinguishing positional isomers.

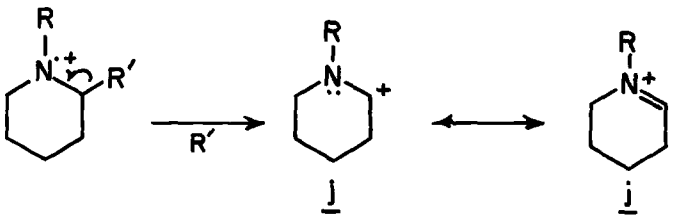

FIG. 17—*Formation of ion* j.

Acknowledgment

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