itself or forms a surface-active coacervate with cetyltrimethylammonium bromide. The equilibration of cetyltrimethylammonium bromide between the bulk solution and the cell wall–solution and solution–air interfaces is fast. The slow drop in surface tension probably resulted from diffusion of nucleic acids with or without bound cetyltrimethylammonium bromide to the surface, which is a slow process.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 18, 1971, from *Temple University School of Pharmacy, Philadelphia, PA 19140*

Accepted for publication January 28, 1972.

Presented in part at the 31st International Congress of Pharmaceutical Sciences, Washington, D. C., September 1971.

Supported in part by a grant-in-aid of research of Temple University.

It is a pleasure to acknowledge the continued advice and assistance given us by Dr. G. D. Shockman and his staff in the Department of Microbiology of Temple University School of Medicine.

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Mass Spectrometric Behavior of Cardiac Steroid Aglycones of the Cardenolide Type

M. B. E. FAYEZ▲ and S. A. R. NEGM

Abstract □ The mass spectra of several cardenolide aglycones are discussed, and the principal modes of fragmentation are outlined. In addition to conventional expulsions of functional groups, the spectra exhibit C_{15}, C_{16}, and C_{17} fragment ions, resulting by elimination of ring D and the side chain as well as ions comprising the latter group with remnants of ring D.

Keyphrases □ Cardenolides—analysis, mass spectroscopy □ Cardiac steroid aglycones, cardenolides—analysis, mass spectroscopy □ Mass spectroscopy—analysis, cardenolides

Constitutionally, the group of cardiac steroid aglycones of the cardenolide type (I) is basically distinguished by an unsaturated γ-lactone side chain (β-oriented) and a β-oriented C-14 hydroxyl group in addition to the ubiquitous C-3 hydroxyl group (mostly β-oriented). The structure is frequently complexed by additional oxygen functions of various types located at various sites of the nucleus. The advent of the technique of mass spectrometry has provided an important tool for structure determination in various classes of natural products (2, 3) but, surprisingly, its application in the group of cardenolides has been extremely limited. Apart from the reported analysis of the spectra for conventional losses of functional groups in several products (4–7), a pattern for fragmentation of the nucleus in digitoxigenin (I) was proposed—without offering a suitable mechanism—by Spiteller (8) and another one was proposed for anhydroafrogenin (II), a C-14 anhydro cardenolide, by Shannon (9).

In the present article, a report is given on the mass spectrometric behavior of certain cardenolide models representing some of the more common types; the proposed fragmentation processes may be found useful in other cases. The molecular ions show up in the spectra, mostly with low intensities, and are always accompanied by abundant ions resulting from the loss of nearly all of the oxygen functional group content: as water from hydroxyl groups, as acetic acid and/or ketene from acetoxyl groups, and as CO (and rarely as CHO) from aldehyde groups. An important feature of frequent appearance is the breakdown of ring A, by a retro-Diels-Alder type of reaction involving loss of butadiene (54 mass units), in the ionized olefin resulting after elimination of the C-3 substituent. This type of breakdown of ring A is possible only with the location of the

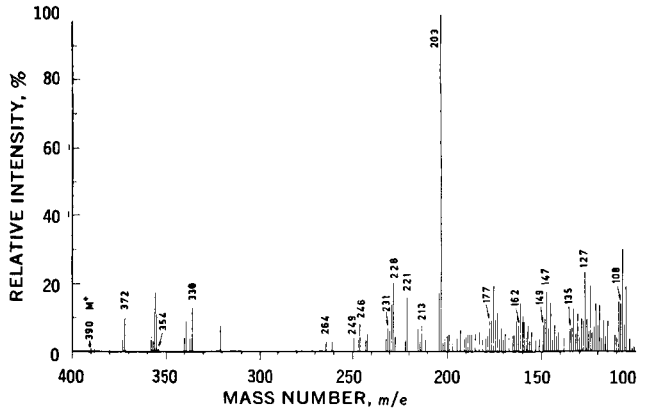


Figure 1—*Mass spectrum of gitoxigenin (V).*

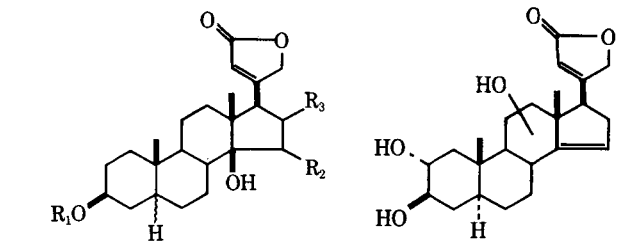
double bond at C-2=C-3 in the formed olefin and is, therefore, evidence for it, regardless of the configuration at C-5 (10). This behavior is sometimes exhibited by ions comprising the intact nucleus but more commonly by ring A-containing fragment ions, *vide infra*. It is not, however, observed in the fragmentation patterns of those products containing hydroxyl groups at C-5 or elsewhere in ring A (in addition to C-3), since the olefinic system resulting by expulsion of such groups would inhibit it.

A study of the mass spectra of several products revealed that the fragmentation of the cardenolide nucleus under electron impact follows a specific pattern which may be of general utility (11). The proposed mechanisms of reactions, as outlined in this report, are speculative; their confirmation—especially those involving specific hydrogen transfers—may be sought by inspection of the spectra of appropriately deuterated models. The major skeletal cleavages of the cardenolide system appear to afford readily distinguishable C_{15}, C_{16}, and C_{17} fragment ions in addition to products comprising the side chain.

DISCUSSION

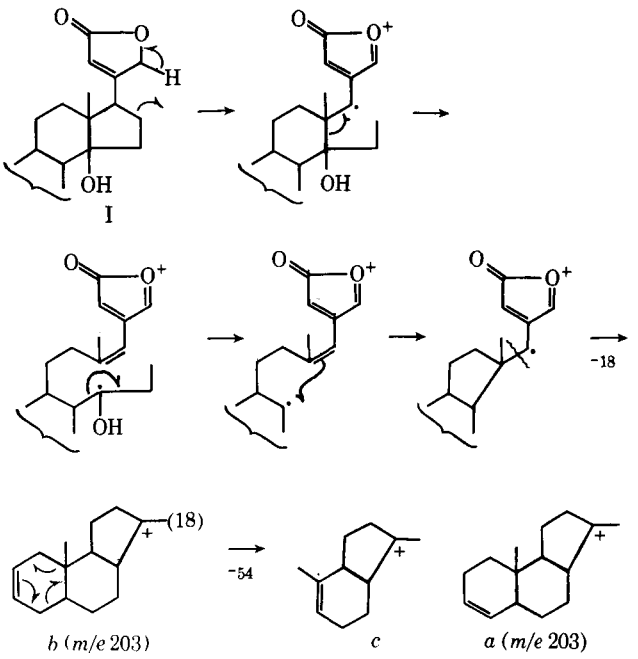
C_{15} Fragmentation Products—One important pathway leads to an ion which appears as a strong or base peak at *m/e* 203 in the published spectra of digitoxigenin (I) (8, 12), 19-desoxoalloglaucotoxigenin diacetate (III) (4), adigenin acetate (IV) (5), and gitoxigenin (V) (*cf.*, Fig. 1). This ion was first assigned the C_{15} constitution *a* (Scheme I) by Spiteller (8), who gave no indication for its origin, in an analysis of the spectrum of somalin (a cymaroside of I) and was later indicated by Ardenne *et al.* (12). The present authors are in complete accord with this assignment except for the location of the double bond which, being the result of expulsion of the C-3 substituent, should be at C-2=C-3 (*b*); this location is evidenced by the subsequent collapse of ring A in a retro-Diels-Alder reaction, giving a peak at *m/e* 149 (*c*) shown in all spectra. One reasonable mechanism is now offered for the genesis of species *b* and involves cleavage of the C-13—C-14, C-8—C-14, and C-13—C-17 bonds. These transitions require that the C-14 hydroxyl group be present and, consequently, that the more stable C-3 function be eliminated in a later stage; the corresponding C-3 oxygenated version is available in the spectra of IV and V (*m/e* 263 and 221, respectively).

The reaction leading to species *b* cannot possibly operate with such constitution as that of anhydroafrogenin (II) (9) due to the effect of the C-14=C-15 double bond which inhibits this type of breakdown. It is, however, represented by strong peaks in the spectrum (Fig. 2) of coroglaucigenin (VI) but by ones with low intensities in the spectra of alloglaucotoxigenin (VII) (4) and its



I: $R_1 = R_2 = R_3 = H$, C-5β
III: $R_1 = Ac$, $R_2 = OAc$, $R_3 = H$, C-5α
IV: $R_1 = Ac$, $R_2 = H$, $R_3 = OCOCH_2CH(CH_3)_2$, C-5β
V: $R_1 = R_2 = H$, $R_3 = OH$, C-5β

II



Scheme I

diacetate (VIII) (4), strophanthidin (IX) (Fig. 3), and nigrescigenin (X) (6), which all exhibit counterparts of species *b* with appropriate mass shifts for the substituents. It appears that the formation of this species is drastically affected by the presence of an aldehyde group on C-19 (VII–X). The spectra reported (7) for sarmentosigenin E acetate (XI) and strogogenin (XII), both of which contain a saturated γ-lactone system engaging C-19 and C-11 (α), also exhibit products (C_{14}) resulting from the same type of fragmentation after the expulsion of carbon dioxide. In all probability, the latter happens[1] at the lactone group between C-19 and C-11, as depicted by the arrows (Scheme II), and also takes place from the molecular ions. The product may be represented by *d*, which is evidenced by peaks at *m/e* 185 from XI and at *m/e* 201 (with an additional OH) and 183 ($-H_2O$) from XII; the spectrum of XI also shows evidence for an anhydro counterpart of species *b* (*m/e* 229).

C_{16} Fragmentation Products—Another course of fragmentation of the nucleus under electron impact, leading in most cases to products with relatively low intensities, takes place by breakdown of the C-13—C-17 bond, with transfer of a hydrogen atom to the departing fragment, and subsequent fission of the allylically activated C-14—C-15 bond. The resulting C_{16} ion may conveniently be represented by *e* (Scheme II), carrying no substituents. It is evidenced by a peak at *m/e* 231 (resulting after elimination of the C-3 function) in the spectra of I and III–V and by peaks with appropriate mass shifts in the spectra of all other products containing substit-

[1] The loss of carbon dioxide postulated (9) to originate from the lactone side chain of anhydroafrogenin (II) has no tangible evidence in the spectra of I and III–X.

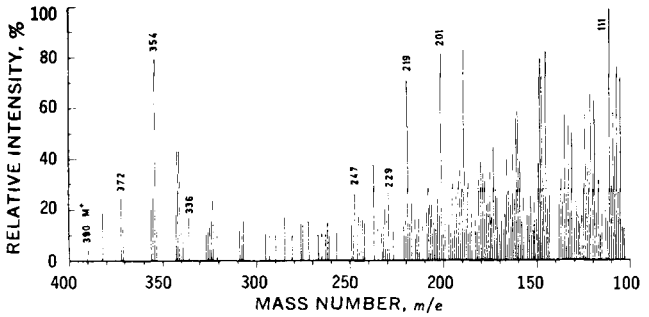


Figure 2—*Mass spectrum of coroglaucigenin (VI).*

uents in, or around, rings A, B, and C. The constitution offered for species *e* is preferred to the alternative oxonium-ion structure (*f*), since subsequent expulsion of water (by elimination of the C-14 hydroxyl) takes place in several cases (substantiated by the metastable ion in the spectrum of V). As might be expected, the spectra of some products exhibit the C-3 oxygenated version of species *e* (V and IX) as well as products resulting from subsequent collapse of ring A by retro-Diels-Alder type of reaction (I, IV, V, and VIII) and loss of carbon monoxide from aldehyde groups (VII and X). The spectra of XI and XII also exhibit the equivalent of species *e* produced after elimination of the lactone grouping (between C-19 and C-11) as CO_2 (leading to structures corresponding to *d*), which is evidenced by peaks at *m/e* 213 and 211, respectively (carrying no oxygen functions in ring A), and at *m/e* 195 and 193, respectively (after further loss of the C-14 hydroxyl).

C_{17} Fragmentation Products—Another mode of fragmentation of the cardenolide nucleus takes place by the partial loss of ring D through cleavage of the C-13—C-17 and C-15—C-16 bonds, with two successive hydrogen atom transfers, leading to ion *g* (Scheme III). This species appears at *m/e* 264 accompanied by two satellite ions (*m/e* 246 and 228), resulting from loss of the remaining hydroxyl groups (*h* and *i*, respectively) in the spectra of both I and V. The published (8) analysis of the spectrum of somalin indicates a specific formulation (*j*) for the peak *m/e* 246. This, in our opinion, is not plausible because of the erroneous location of the double bond in ring A and the unstabilized charge assignment. Moreover, the expected breakdown of ring A by a retro-Diels-Alder type of reaction happens only from the ion at *m/e* 228 and not from that at *m/e* 246, which may indicate that the latter ion retains the more stable C-3 hydroxyl (*h*). Species *g* or its companions resulting by dehydration and/or expulsion of carbon monoxide are represented (with appropriate mass shifts) in the spectra of all other cardenolides, with the exception of VI. Of special significance and possible structural utility is the demonstration of this type of fragmentation in the spectra of those compounds which are oxygenated at C-15 (III, VII, and VIII), leading to fragment ions still retaining this substituent. Compounds II and XII also exhibit this mode of fragmentation by giving ions of species *i* (less one carbon atom due to loss of carbon dioxide from the lactone system) and corresponding in constitution to *d* after the loss of all hydroxyl content.

Cardenolide Side Chain—Evidence for the formation of ions comprising the lactone side chain in addition to carbons 16 and 17 is available in the appearance of appreciable peaks at *m/e* 111 in the spectra of all cardenolides carrying no substituent on C-16 and at *m/e* 127 in that of V (with a hydroxyl function on C-16). The con-

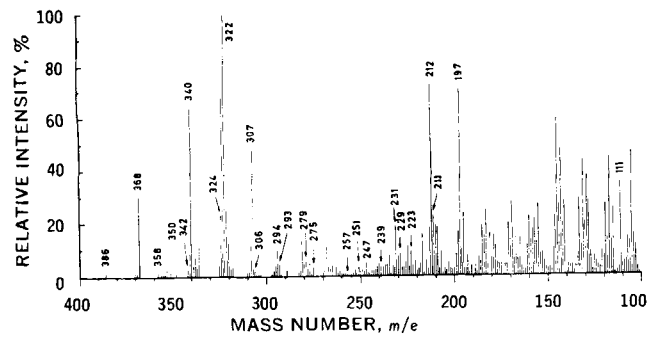
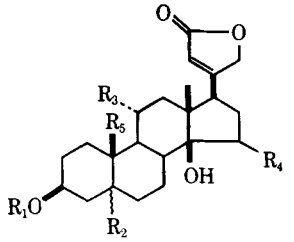
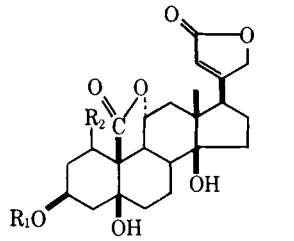


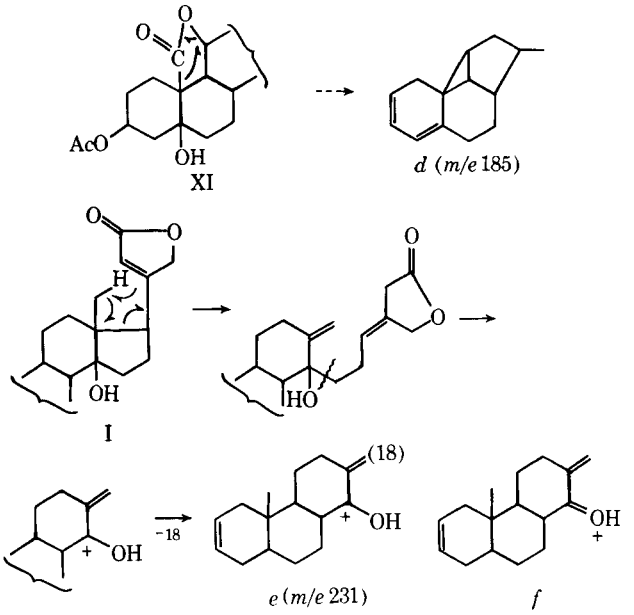
Figure 3—*Mass spectrum of strophanthidin (IX).*



VI: $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = CH_2OH$, C-5α
VII: $R_1 = R_2 = R_3 = H$, $R_4 = OH$, $R_5 = CHO$, C-5α
VIII: $R_1 = Ac$, $R_2 = R_3 = H$, $R_4 = OAc$, $R_5 = CHO$, C-5α
IX: $R_1 = R_3 = R_4 = H$, $R_2 = OH$, $R_5 = CHO$, C-5β
X: $R_1 = R_4 = H$, $R_2 = R_3 = OH$, $R_5 = CHO$, C-5β



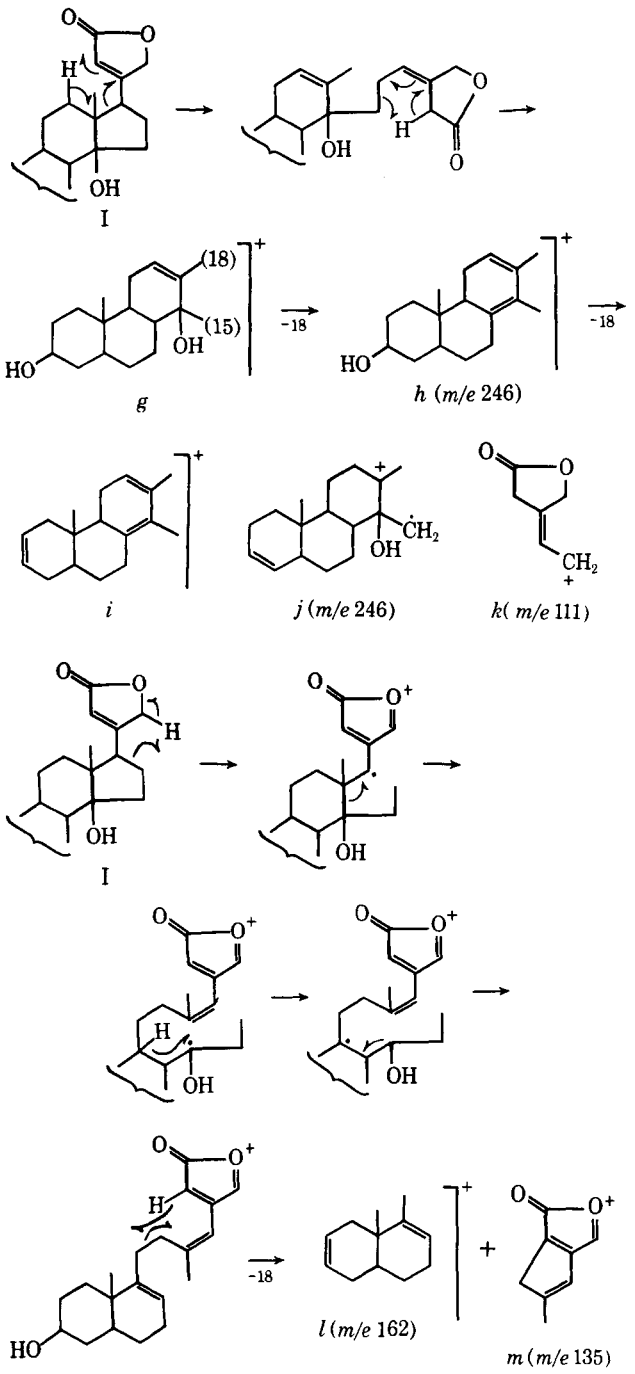
XI: $R_1 = Ac$, $R_2 = H$
XII: $R_1 = H$, $R_2 = OH$



Scheme II

stitution of the resulting ion species is conveniently represented by *k* (Scheme III), the genesis of which was reasonably accounted for by Shannon (9) during a study of the fragmentation of anhydroafrogenin (II). The remainder of the molecule does not give rise to an ionized fragment with the appropriate *m/e* value in any of the spectra, nor is there any evidence for the expulsion of carbon dioxide from the side chain of I and III–X, which is in contrast to the reported (9) behavior of II. The spectra of XI and XII exhibit this species, but that of IV shows no evidence for it, presumably because the double bond formed after the facile expulsion (5) of isovaleric acid from C-16 inhibits this type of fragmentation.

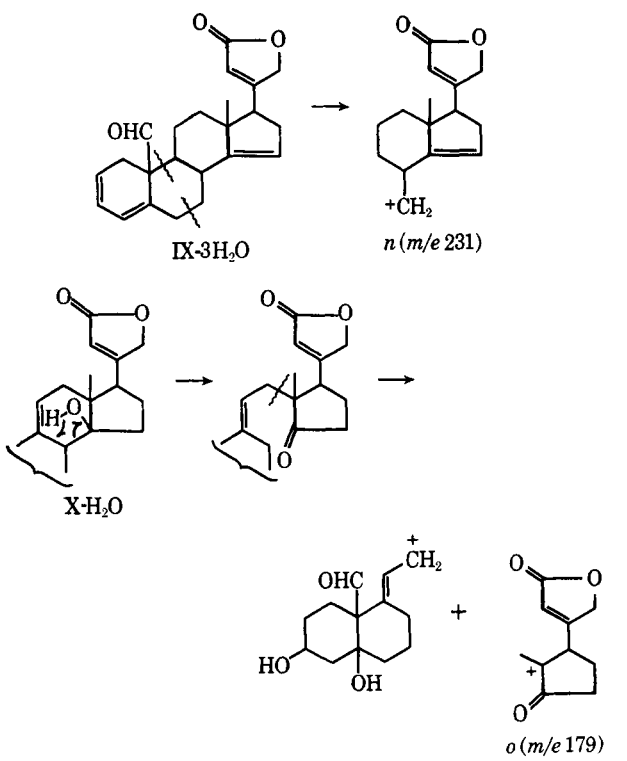
An additional feature in the spectra of I, III, and V is the presence of a number of peaks in the low mass range with appreciable intensities; these may be attributed to yet another mode of fragmentation. This is depicted by the arrows in Scheme III and results in the total loss of carbons 14, 15, and 16 and the formation of two ions (*l* and *m*), each of which may carry the charge. Species *l* appears in the three spectra at *m/e* 162 and is accompanied by the C-3 oxygenated counterpart and by products resulting from the



Scheme III

loss of 15 (CH_3) and 54 (ring A) mass units. Species *m* shows up at *m/e* 135 and may undergo loss of carbon dioxide by an ambiguous reaction.

The spectra of strophanthidin (IX) and nigrescigenin (X) contain peaks with low abundance at *m/e* 231 and at *m/e* 247 and 247 − H_2O, respectively, which may be construed as evidence for a special fragmentation course initiated by the olefinic system formed by loss of the hydroxyl groups on C-5 and C-14. The resulting ion, formed by cleavage of the allylically activated C-6—C-7 and the highly substituted C-9—C-10 bonds, may be formulated as *n* (Scheme IV). No such behavior may be expected in the spectra of the analogously constituted compounds, XI and XII, which, after the loss of CO_2 from lactone between C-19 and C-11, cannot be readily cleaved across ring B. The presence of the C-11 hydroxyl group in X may also be responsible for another reaction type induced by the ring C olefinic bond resulting by expulsion of this substituent. Thus, transfer of hydrogen from the C-14 hydroxyl group to C-8 and cleavage of the allylically activated C-12—C-13



Scheme IV

bond leads to species *o*, which appears as the strongest peak (*m/e* 179) in the high mass range of the spectrum (6) of nigrescigenin (X). The rest of the molecule may also carry the charge, as evidenced by peaks with low abundance resulting after losses of H_2O (*m/e* 223), H_2O + CO (*m/e* 195), and 2 H_2O (*m/e* 205).

It is generally emphasized that the presence of a C-14 hydroxyl group is a prerequisite for the display of the previously discussed principal modes of fragmentation of the cardenolide nucleus; the C-14 anhydro compounds—like anhydroafrogenin (9) (II)—do not exhibit such reactions, undoubtedly because of the inhibiting effect of the C-14=C-15 ethylenic linkage. The presence of a carbonyl group on C-19 (as in VII-X) does not seem to induce any special modes of fragmentation [*e.g.*, of the McLafferty (13) type], probably because of its expulsion with considerable facility as a primary reaction. The utility of the mass spectra becomes considerably reduced with the increased complexity of structure—such as by multiple hydroxyl content as in ouabagenin—since the nuclear fragmentation is diversely affected by the olefinic content of the dehydration products and the fragment ions are accompanied by multiple versions of such products, which all renders the spectrum too complex for reliable analysis.

EXPERIMENTAL

Materials—The compounds used in this study were obtained either by isolation from natural sources or as gifts.

Mass Spectra—These were made employing an instrument (AEI MS9) operated with a source temperature of 250° and an ionizing voltage of 70 ev.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 13, 1971, from the *National Research Centre, Dokki, Cairo, Egypt.*

Accepted for publication January 19, 1972.

The authors are deeply indebted to Professor Dr. T. Reichstein (University of Basel) for gifts of authentic samples of some compounds and to Professor A. V. Robertson (University of Sydney) for the mass spectral measurements.

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Effect of Age on Ultracentrifugal Stability of Liquid Petrolatum–Water Emulsions

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Abstract □ The effect of aging over 135 days on the rate of separation of oil from 50% liquid petrolatum–50% water emulsions with both 0.2 and 0.4% sodium dodecyl sulfate (based on the water) was determined in an ultracentrifuge at 25° and 39,460 r.p.m. The concentration of sodium dodecyl sulfate in the equilibrium aqueous phase was also determined. The stability of the emulsion with 0.2% sodium dodecyl sulfate decreased for the first 25 days and thereafter increased with time, with a qualitative change in the mode of loss of oil after 135 days. There was no change in the stability of the emulsion with 0.4% sodium dodecyl sulfate over the same time period. The results with the 0.2% sodium dodecyl sulfate emulsion can be rationalized in terms of an initial increase in drop size with an accompanying desorption of sodium dodecyl sulfate and a decrease in stability, followed by coverage of a greater fraction of the remaining oil–water interface by adsorbed sodium dodecyl sulfate from the more concentrated solution of sodium dodecyl sulfate resulting from the initial desorption.

Keyphrases □ Liquid petrolatum–water emulsions—effect of age on stability, ultracentrifugation □ Stability of liquid petrolatum–water emulsions—age effect □ Emulsions, liquid petrolatum–water—effect of age on stability, ultracentrifugation □ Ultracentrifugation—determination of stability of liquid petrolatum–water emulsions

Since the stability of emulsions is of great importance in the pharmaceutical, food, and cosmetic industries, studies elucidating the mechanism of the demulsification process or seeking an accelerated test for determining stability are very useful. In a recent review, Garrett (1) referred to numerous attempts that have been made to characterize emulsions in terms of their drop size or interfacial area, particularly in terms of the change in these quantities with time, as well as by means of the rate of separation in an ultracentrifuge of bulk oil from freshly prepared emulsions. While there have been many studies of drop size and surface area as a function of time (2–10), only limited information is available as to the effect of the age of the emulsion on the rate at which oil separates. Merrill (11) found that the amount of oil separated from a relatively unstable butyl phthalate–water–sodium laurate emulsion in a basket centrifuge seemed to vary linearly with time, and the rate *increased* from 0.03 to 0.33 ml./min. over 4 days. Garrett (12) reported a small *decrease* in the rate of separation of oil from a toluene–water–polyoxyethylene stearate emulsion in an ultracentrifuge after 11 days of aging. The present authors (13) found that the ultracentrifugal rate of separation of oil from a liquid petrolatum[1]–water–sodium dodecyl sulfate (I) emulsion increased by about one-third after the emulsion stood undisturbed for 18 days.

The present paper reports data on the rate of separation of oil at 25° in an ultracentrifuge at 39,460 r.p.m. from 50% liquid petrolatum–50% water–I emulsions with the same drop size distribution but containing either 0.2 or 0.4% I on the basis of the aqueous phase after different times of standing up to 135 days. From previous work (8, 14, 15) it was known that initially in such an emulsion with 0.2% I, the oil–water interface would not be completely covered by adsorbed I; whereas at 0.4% initial concentration of I in the aqueous phase, adsorption at the interface would have already reached the saturation limit. The observed differences in the effect of age on the ultracentrifugal stability in the two cases can be explained in terms of this difference between the two systems. However, caution must be exercised in extrapolating the conclusions based on ultracentrifugal data to free-standing emulsions, since the latter consist of spherical

[1] Nujol.