TABLE V

Fraction	Band color
9M1	Red-orange
9M2	Yellow
9M3	Yellow
9M4	Yellow
9M5	Yellow
9M6	Yellow
9M7	Purple
9M8	Red
9M9	Pink
9M10	Orange
9M11	Purple
9M12	Orange-brown (remaining bands)

TABLE VI

Fraction	Band color
9M12A	Orange
9M12B	Orange
9M12C	Purple
9M12D	Yellow
9M12E	Brown
9M12F	Yellow
9M12G	Yellow
9M12H	Brown
9M12I	Brown

Isolation of Spinochrome from *E. calamaris*.—A 700-g batch of spines was processed for crude pigments as described above. Essentially the same array of pigments that was found in *E. diadema* was displayed. The only significant differences that were noted are (1) 2,5-dihydroxy-3-ethylbenzoquinone could not be detected and (2) fraction 1 exhibited seven pigments instead of one for *E. diadema*.

Fraction 1 was chromatographed on a thin-layer plate with benzene and separated into a red band (1A), three yellow bands (1B, 1C, and 1D), a red band (1E), a yellow band (1F), and a red-orange band (1G). Fraction 1E proved to be identical with **2-hydroxy-3-acetylnaphthazarin** (1). Fraction 1F was too small to crystallize, but its ultraviolet spectrum and R_f value were identical with those of **2-hydroxy-6-ethyljuglone** (2). Fraction 1G crystallized from isooctane to give 0.25 mg of **2-hydroxy-6-ethylnaphthazarin** (3), mp 204–204.5°.

Conversion of 2-Hydroxy-6-acetylnaphthazarin to 2-Hydroxy-6-ethylnaphthazarin.—Two milligrams of the natural pigment, 2-hydroxy-6-acetylnaphthazarin, was carefully methylated with diazomethane and after purification by tlc the resulting 2-methoxy-6-acetylnaphthazarin, red crystals from isooctane, mp 185–186° dec, was dissolved in 2 ml of methanol and *ca.* 1 mg of sodium borohydride was added (red color immediately discharged). The yellow solution was acidified with hydrochloric acid (red color slowly returns) and the reduction product was extracted with benzene and separated from unreduced material by thin layer chromatography. The 2-methoxy-6-ethylnaphthazarin, which was not isolated, was hydrolyzed to 2-hydroxy-6-ethylnaphthazarin by a 2-min boiling in 1 ml of ethanol–12 *N* hydrochloric acid (1:1). The product was identified as 2-hydroxy-6-ethylnaphthazarin by comparison of its ultraviolet spectrum and R_f value (tlc) with those of an authentic sample. On a thin layer plate of acid-treated, deactivated silica gel, 2-hydroxy-6-ethylnaphthazarin moves faster than the 7-ethyl isomer in carbon tetrachloride.

Synthesis of 2,5-Dihydroxy-3-ethylbenzoquinone.—The quinone was prepared using a modified procedure of Jones and Shonle[24] for 2,5-dihydroxybenzoquinone. A stirred mixture of 40 mg of ethylhydroquinone in 1 ml of 60% aqueous sodium hydroxide was treated with 0.5 ml of 30% hydrogen peroxide. The temperature rose to 45° and was maintained at 45–50° for 2 hr during which time the mixture became a thick paste. It was poured onto ice and acidified with hydrochloric acid. The product was extracted from the resulting yellow solution with ether and purified by vacuum sublimation. After crystallization from benzene 13 mg of 2,5-dihydroxy-3-ethylbenzoquinone (27%) was obtained as orange prisms, subliming at 130–145° without melting. The synthetic quinone was identical in every respect (nmr, infrared and ultraviolet spectra, and R_f values is two systems) with the natural pigment.

Acknowledgment.—We should like to thank Mr. Lester C. Zukeran of the Hawaii Institute of Marine Biology for collecting the animals and Dr. D. Becher and Professor C. Djerassi for determining the mass spectra. The authors are grateful to Miss Glory Barayuga for invaluable technical assistance. This investigation was supported by a PHS Grant GM 10413 from the Institute of General Medical Sciences, Public Health Service.

(24) R. G. Jones and H. A. Shonle, *J. Am. Chem. Soc.*, **67**, 1034 (1945).

Mass Spectrometry in Structural and Stereochemical Problems.[1] CXI. The Mass Spectrometric Fragmentation of Substituted Naphthoquinones and Its Application to Structural Elucidation of Echinoderm Pigments[2]

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The mass spectra of a series of acetyl, and higher substituted naphthoquinones are presented. The generalizations set out by Beynon and Williams and by Williams and co-workers have been expanded by considering the fragmentation patterns characteristic for acetylnaphthoquinones. The acetyl function surpasses the hydroxy and methoxy function in its ability to direct fragmentation upon electron impact. Its location in the quinoid or benzenoid moiety of the molecule and in the vicinity of various other substituents yields different spectra, which can be used successfully for structural elucidation of unknown pigments of this group.

Naphthoquinone pigments occur widely in nature among higher plants and microorganisms, yet in the animal kingdom this class of compounds has been encountered only in echinoderms. Even within this phylum only the echinoids (sea urchins) have been the prime producer of these substances. There they occur as structural pigments (spinochromes) and in the eggs, ovaries, and perivisceral fluid of the animals (echinochromes). The first research in this field dates back to 1885,[4] but it was more than 50 years later when a pure,

(1) Paper CX: A. M. Duffield, S. D. Sample, and C. Djerassi, *Chem. Commun.*, 193 (1966).

(2) Supported in part by Grants No. GM-11309 and GM 10413 (PHS).

(3) Recipient of a NATO Postdoctoral Fellowship.

(4) C. A. MacMunn, *Quart. J. Microscop. Sci.*, **25**, 469 (1885).

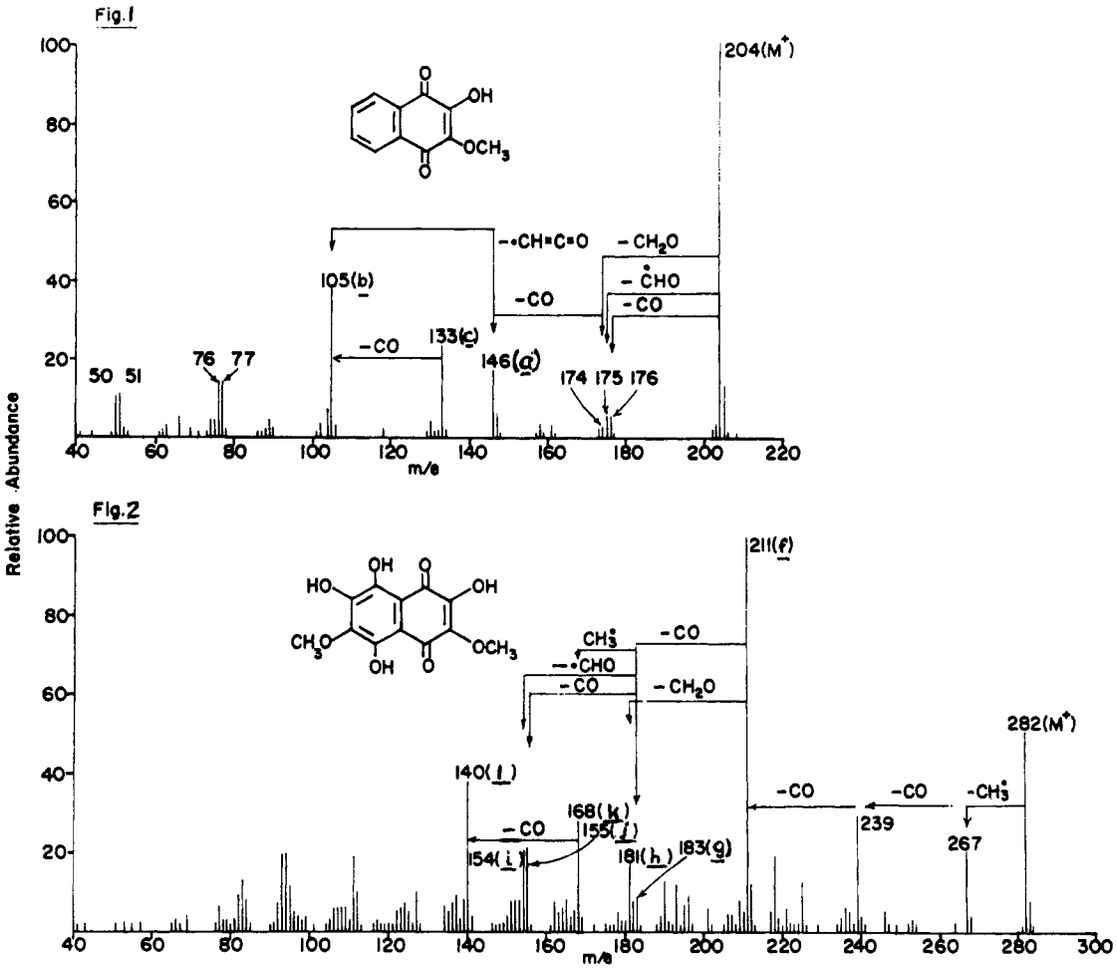
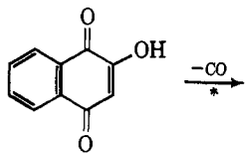


Figure 1.—Mass spectrum of 2-hydroxy-3-methoxynaphthoquinone.

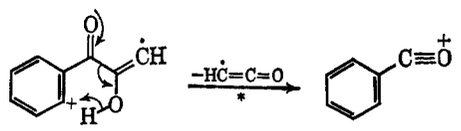
Figure 2.—Mass spectrum of 2,7-dihydroxy-3,6-dimethoxynaphthazarin.

crystalline pigment was isolated and identified.[5] Some of the problems encountered with these compounds were stated by Thomson:[6] "The identification of these pigments is hampered by analytical difficulties and inconvenient physical properties, and it may be that some of the pigments at present given different distinguishing letters, are in fact identical." This prediction was shown to be correct by Scheuer and co-workers,[7] who isolated spinochrome M (later found to be identical with A[8]) from six sea urchin species. In spite of this advance, research on polyhydroxy naphthoquinones with their high oxygen-to-carbon ratios continues to be plagued by the inadequacy of combustion data. This difficulty, coupled with the small amounts (10^{-6}–10^{-5}%) in which some of these compounds are isolated from the animals, has prompted us to use mass spectrometry as a tool for structural elucidation. As an aid to the interpretation of the mass spectra, a number of compounds were synthesized,[9] whose fragmentation patterns will be discussed along with those of the natural pigments.

Hydroxynaphthoquinones.—The mass spectra of a number of hydroxylated naphthoquinones were investigated recently by Williams and co-workers,[10] who demonstrated that their electron-impact-induced fragmentation follows principally the pathways established by Beynon and Williams[11] for naphthoquinone itself. 2-Hydroxynaphthoquinone (1, lawsone) exhibits in addition a highly characteristic hydrogen-rearrangement peak[10] (see a → b), which has been shown to be



1, *m/e* 174



a, *m/e* 146 b, *m/e* 105

present also in its 3-substituted derivatives. In the spectrum (Figure 1) of 2-hydroxy-3-methoxynaphthoquinone (2) the fragments characteristic of 2-hydroxy-

(5) R. Kuhn and K. Wallenfels, *Chem. Ber.*, **72**, 1407 (1939).

(6) R. H. Thomson, "Naturally Occurring Quinones," Butterworth and Co. (Publishers) Ltd., London, 1957, pp 128–140.

(7) C. W. J. Chang, R. E. Moore, and P. J. Scheuer, *J. Am. Chem. Soc.*, **86**, 2959 (1964).

(8) C. W. J. Chang, R. O. Moore, and P. J. Scheuer, *Tetrahedron Letters*, 3557 (1964).

(9) For experimental details see C. W. J. Chang, R. E. Moore, R. Ogata, I. Singh, and P. J. Scheuer, forthcoming publication.

(10) J. H. Bowie, D. H. Cameron, and D. H. Williams, *J. Am. Chem. Soc.*, **87**, 5094 (1965).

(11) J. H. Beynon and A. E. Williams, *Appl. Spectry.*, **14**, 156 (1960).

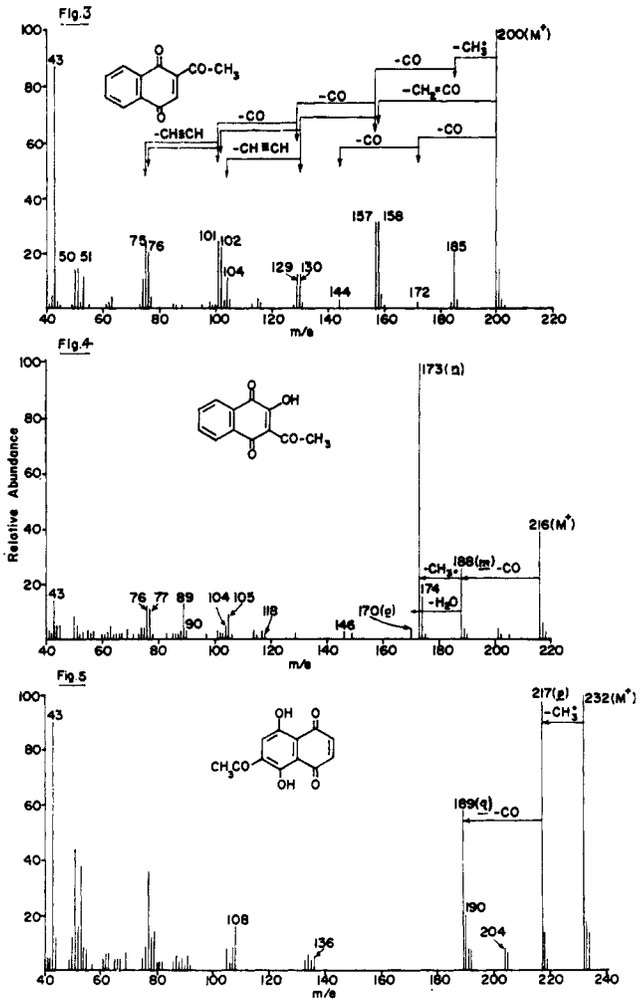
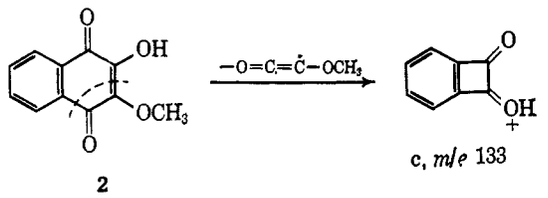


Figure 3.—Mass spectrum of 2-acetylnaphthoquinone.

Figure 4.—Mass spectrum of 3-acetyl-2-hydroxynaphthoquinone.

Figure 5.—Mass spectrum of 6-acetylnaphthazarin.

naphthoquinone[10] are superimposed upon peaks demonstrating the presence of the methoxy group (*e.g.*, M − 29, *m/e* 175) and its proximity to the hydroxyl group in the quinoid part of the molecule (c, *m/e* 133).[12]



2 c, *m/e* 133

Surprising, however, is the complete absence of a M − 15 ion in 2.

The influence of hydrogen bonding on the fragmentation of the molecule under electron impact is illustrated with 2,5,8-trihydroxynaphthoquinone (naphthopurpurin). While the breakdown under electron bombardment generally follows the rules established by Williams,[10] it is interesting to note that the hydrogen rearrangement typical of 2-hydroxynaphthoquinone (see a → b) is nearly suppressed.

(12) Although no metastable ion accompanies this transition, a similar fragmentation is noted in the mass spectrum of 2,3-dihydroxynaphthoquinone where O=C=C̈—OH is lost from the molecular ion with an accompanying metastable ion at *m/e* 93.4 ($133^2/190$ = 93.1).

Hydrogen rearrangement fragmentations are completely absent in the spectrum (Figure 2) of 2,7-dihydroxy-3,6-dimethoxynaphthazarin (**3**), the major pigment from the spines of the asteroid *Acanthaster planci* Linn.[13] As outlined in Scheme I, the primary loss of methyl (d, *m/e* 267) in **3** is followed by stepwise elimination of CO, which gives rise to the peaks at *m/e* 239 (e), 211 (f), 183 (g), and 155 (j). As in **2**, formaldehyde is lost only to a small extent from the molecular ion. Considerably more important is its elimination from the abundant ion of mass 211 to form *m/e* 181 (h) as substantiated by the metastable ion at *m/e* 155.3 ($181^2/211$ = 155.3). The peaks at *m/e* 154 (i) and 155 (j) indicate that the methoxy groups are located in different rings. This evidence is reconfirmed by the loss of methyl from *m/e* 183 (g) to form *m/e* 168 (k), which subsequently eliminates carbon monoxide to yield l (*m/e* 140). Metastable ions at *m/e* 131.3 ($155^2/183$ = 131.3) and 116.6 ($140^2/168$ = 116.6) support these transitions.

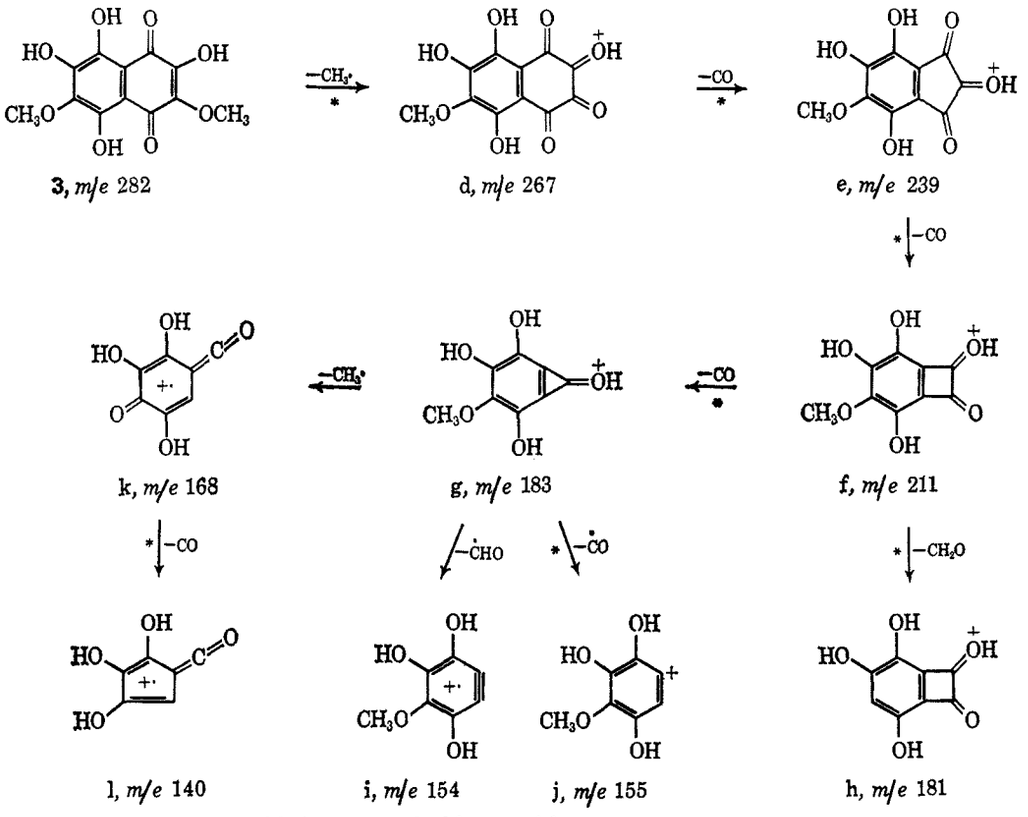
In the absence of ^{18}O or ^{13}C labeling, the order of carbon monoxide expulsion from the molecule is depicted in an arbitrary manner for illustrative purposes only. The same reservation applies to most other CO eliminations in the present article. It should be noted at the outset that the various representations attributed to fragment ions in the present article are employed in an illustrative manner to aid in generalizations suitable for structure work rather than to define unequivocally established structures.

Acetylnaphthoquinones.—2-Acetylnaphthoquinone (**4**) breaks down in a well-defined way under electron impact to yield a spectrum (Figure 3) with highly characteristic "doublet" structure. Possible fragmentation paths are summarized in Scheme II, which demonstrates two main avenues of disintegration. In the first, methyl is lost in the initial step (*m/e* 185), and successive elimination of carbon monoxide and acetylene gives rise to the peaks at *m/e* 157, 129, 101, and 75. While loss of CO occurs only to a very small extent from the molecular ion ($M^+ \rightarrow$ *m/e* 172 → *m/e* 144), the second major fragmentation is started by expulsion of ketene to form naphthoquinone (*m/e* 158), which then breaks down further as outlined by Beynon.[11] Metastable ions accompany most transitions. Finally, it should be noted that the acetyl function can be lost from the quinoid ring with charge retention to form the second most abundant ion of mass 43.

Introduction of an additional hydroxyl function adjacent to the acetyl group changes the picture completely. In the spectrum (Figure 4) of 3-acetyl-2-hydroxynaphthoquinone (**5**) ketene is lost from the molecular ion to only a minor extent (*m/e* 174) since the acetyl carbonyl is strongly hydrogen bonded to the adjacent hydroxyl. The expulsion of CO is now favored as the initial step (m, *m/e* 188), followed by the loss of a methyl radical to form the very stable ion n (*m/e* 173). Ions at lower masses are not very abundant, but the complete set of peaks (*m/e* 146, 118, 105, 90, 89, 77, and 76) for 2-hydroxynaphthoquinone[10] (**1**) can be detected. It is interesting to note in this case the insignificant contribution of *m/e* 43 to the total ionization, stressing again the hydrogen-bonded nature of the acetyl carbonyl and the preferential formation and high

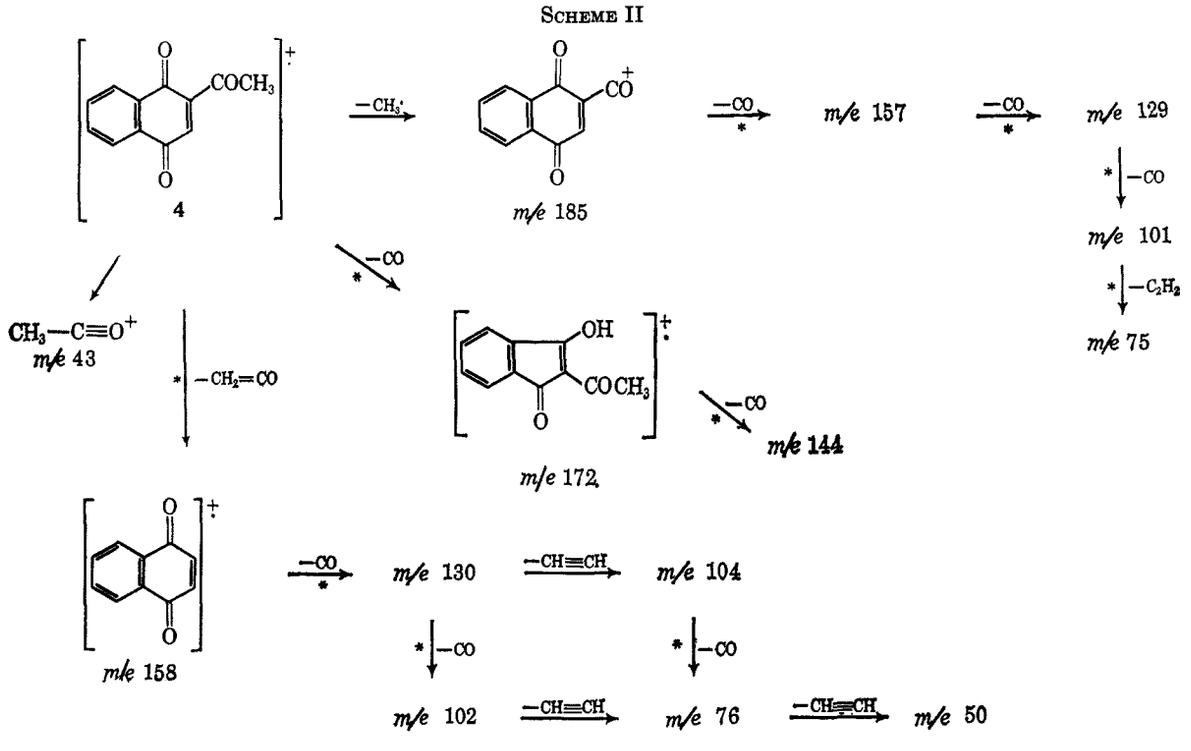
(13) H. Singh, R. E. Moore, and P. J. Scheuer, *Science*, in press.

SCHEME I[a]



[a] Transitions supported by a metastable ion are marked by asterisks.

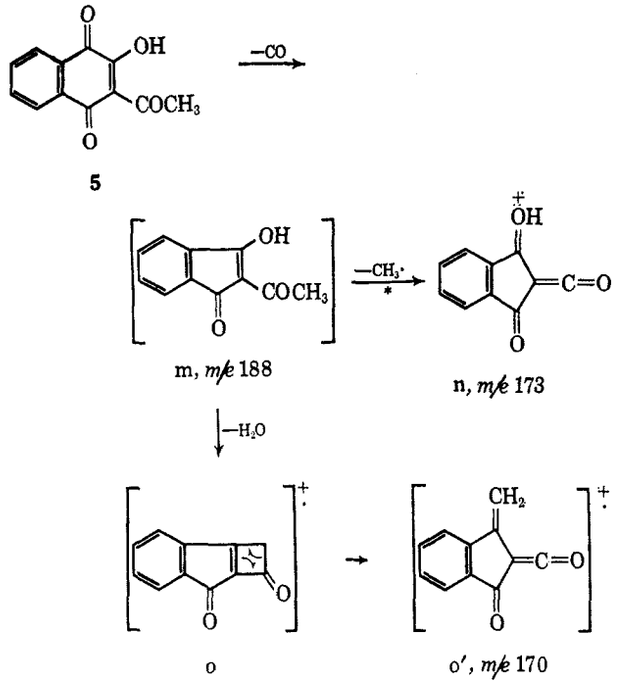
SCHEME II



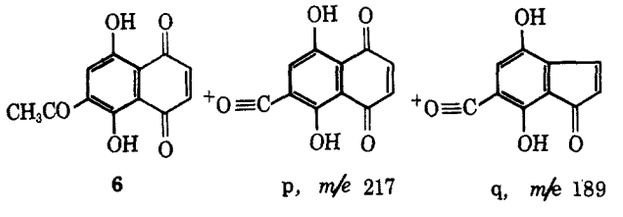
stability of an ion such as n. Water can be lost from m to yield *m/e* 170 (o). Although ion o does not constitute a major fragment, its appearance is characteristic for the vicinal nature of acetyl and hydroxyl functions, as will be further shown below.

Loss of ketene is less favored (see Figure 5) in 6-acetylnaphthazarin (6, containing some 1,4,5,8-tetrahydroxy-2-acetylnaphthalene as an impurity) than in 4, since the acetyl substituent is predominantly located on the aromatic ring.[14] It gives rise to the peak at *m/e* 190 (naphthazarin), which breaks down further under electron impact to generate the familiar fragments at *m/e* 136 and 108,[10] which are characteristic for the benzenoid part of the molecule. The predominant

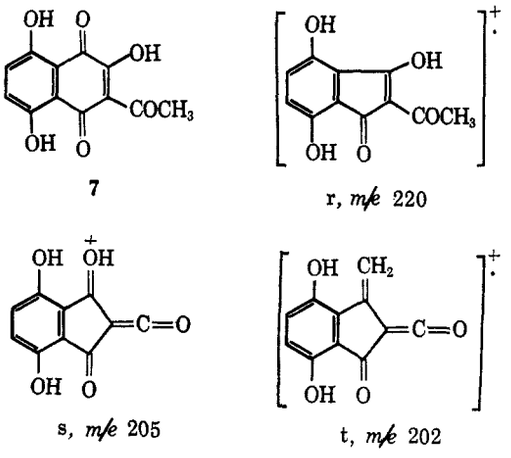
(14) This assignment is based on nmr evidence which is discussed in a publication by R. E. Moore and P. J. Scheuer, *J. Org. Chem.*, 31, 3272 (1966).



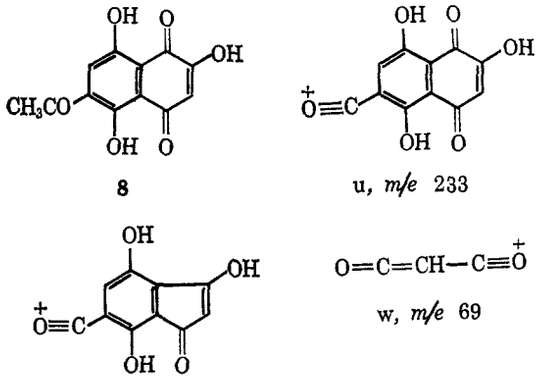
fragmentation of this compound is initiated by loss of a methyl radical, followed by expulsion of carbon monoxide. Metastable ions at m/e 203.0 ($217^2/232$ = 203.0) and m/e 164.5 ($189^2/217$ = 164.5) accompany the formation of the ions at m/e 217 (p) and m/e 189 (q). An M − 28 species is present in the spectrum, though rather small, and m/e 43 is now again responsible for an outstanding peak.



With this material as background, the spectra of three minor pigments (**7**–**9**) from the spines of the echinoid *Echinothrix diadema* Linn.[15] were examined. Two pigments had an electronic spectrum characteristic of a naphthazarin, whereas the other pigment exhibited a typical juglone spectrum. A preliminary nmr examination revealed the presence of an acetyl function in all three pigments. For the less polar of the two naphthazarin derivatives (**7**) disintegration upon electron impact starts with the elimination of CO (r, m/e 220, 12% relative intensity), which looses a methyl radical to form the homolog of n, at m/e 205 (s, 75% relative intensity). Expulsion of ketene, less pronounced in the naphthazarin series as compared with the corresponding naphthoquinone derivatives, is further suppressed in this substance (m/e 206, 11% relative intensity, including the isotope peak of s), while loss of water from M − 28 constitutes an outstanding peak at m/e 202 (t, 37% relative intensity), indicating vicinal acetyl and hydroxyl functions on the naphthazarin skeleton. The substance is therefore 3-acetyl-2-hydroxynaphthazarin (**7**). A completely different



fragmentation pattern (Figure 6) is observed for the more polar naphthazarin isomer (**8**). Formation of m/e 205 (v) is here accomplished by the initial loss of a methyl radical from the molecular ion, which gives rise to the peak at m/e 233 (u). Appropriate metastable ions at 218.9 ($233^2/248$ = 218.9) and 180.4 ($205^2/233$ = 180.4) support this pathway of disintegration. Carbon monoxide is eliminated to a minor extent from the molecular ion. The complete absence of a fragment at m/e 202 (t) locates the acetyl and the additional hydroxyl group in different rings. Noteworthy is the peak at m/e 69 (w), which Williams, *et al.*,[10] have found to be characteristic of all naphthoquinone derivatives containing the (OCCCO) unit. Further chemical evidence[15] shows **8** to be 6-acetyl-2-hydroxynaphthazarin.



The need for additional information becomes more important for the third isomer (**9**) which contains the juglone chromophore. Its mass spectrum (Figure 7) is identical with the one observed for **8**, as far as the pathway of ion formation is concerned, and structural assignments have to be based on changes in relative intensity of the few peaks exhibited. The extraordinarily strong M − 15 peak (x, m/e 233) excludes the placement of the acetyl function in the quinoid moiety together with an additional OH group. The very small peak at m/e 230 (loss of water from the molecular ion) supports its location next to a hydroxyl. The structure of **9** has been shown to be 6-acetyl-2,7-dihydroxyjuglone by synthesis.[16]

(15) Accompanying paper: R. E. Moore, H. Singh, and P. J. Scheuer, *J. Org. Chem.*, **31**, 3645 (1966).

(16) Accompanying paper: R. E. Moore, H. Singh, C. W. J. Chang, and P. J. Scheuer, *ibid.*, **31**, 3638 (1966).

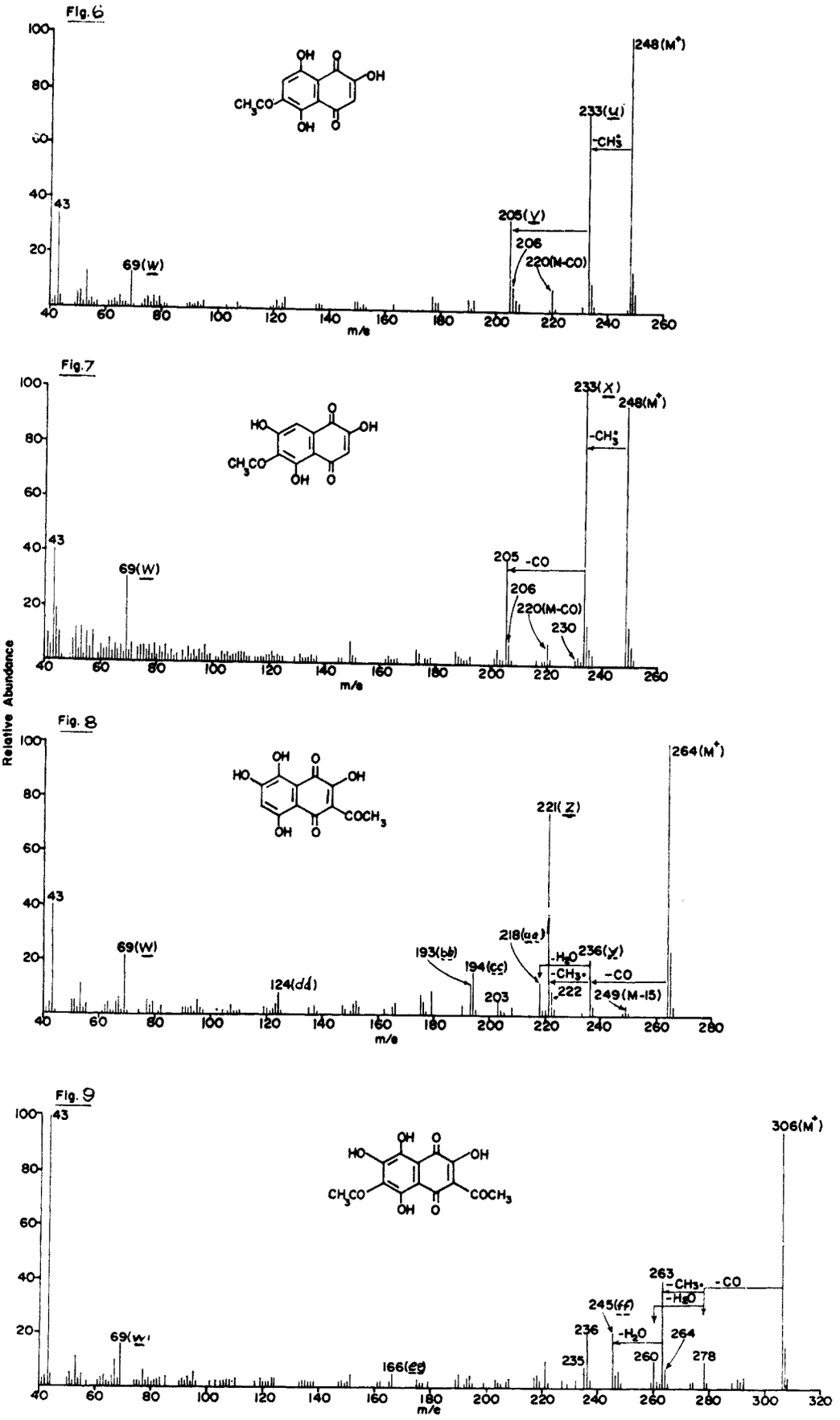
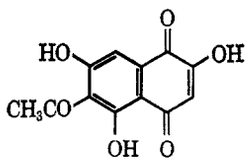


Figure 6.—Mass spectrum of 6-acetyl-2-hydroxynaphthazarin.

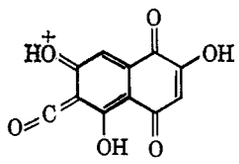
Figure 7.—Mass spectrum of 6-acetyl-2,7-dihydroxyjuglone.

Figure 8.—Mass spectrum of 3-acetyl-2,7-dihydroxynaphthazarin.

Figure 9.—Mass spectrum of 3,6-diacetyl-2,7-dihydroxynaphthazarin.

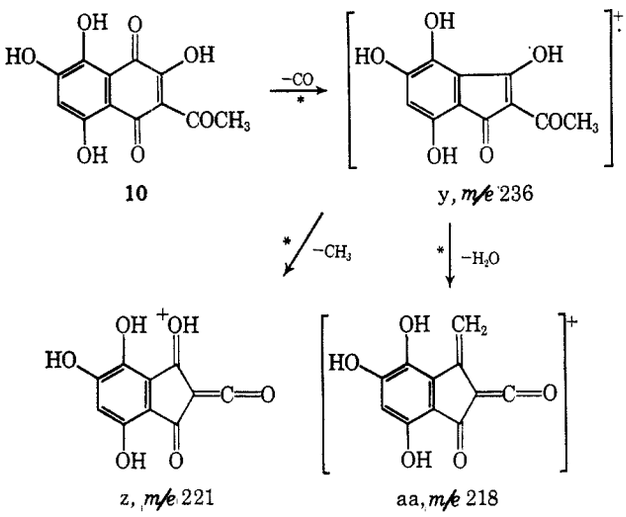


9



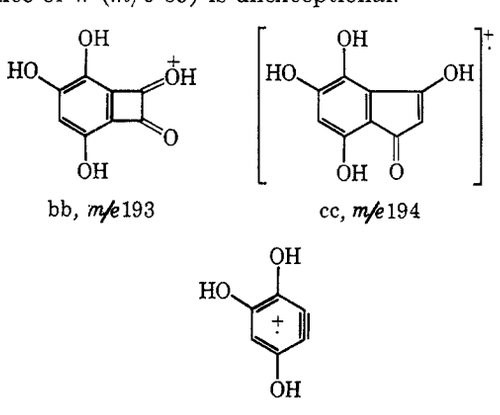
x, *m/e* 233

The spectrum (Figure 8) of 3-acetyl-2,7-dihydroxynaphthazarin (**10**, spinochrome A) substantiates again the previous conclusions. Vicinal hydroxyl and acetyl functions in the quinoid moiety effect expulsion of CO (y, *m/e* 236) as the first step, followed by loss of methyl to form ion z at *m/e* 221 in analogy with n and s, as well as by elimination of water to give rise to aa at *m/e* 218 (analogous to o and t). Expulsion of ketene



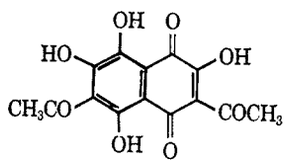
10 y, *m/e* 236 z, *m/e* 221 aa, *m/e* 218

(*m/e* 222) and elision of acetyl with charge retention (*m/e* 43) proceed to the anticipated extent. Carbon monoxide can be lost from *m/e* 221 and 222 to yield *m/e* 193 (bb) and 194 (cc). All transitions mentioned are supported by metastable ions; however, there is an additional metastable peak at *m/e* 159.5 ($194^2/236 = 159.5$), which indicates the partial formation of cc by loss of ketene from M − CO (y). Finally, *m/e* 124 (dd) supports placement of the three hydroxyl functions in the benzenoid part of the molecule, while the presence of w (*m/e* 69) is unexceptional.[10]

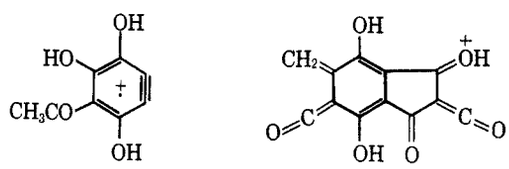


bb, *m/e* 193 cc, *m/e* 194 dd, *m/e* 124

Introduction of an additional acetyl function in position 6 of **10** yields 3,6-diacetyl-2,7-dihydroxynaphthazarin (**11**, Figure 9).[17] The fragmentation of this highly symmetrical compound repeats to a first approximation the pattern outlined for **10**, with peaks shifted 42 mass units to higher masses. The exceptional intensity of *m/e* 43 can be easily explained by the presence of two acetyl groups in the molecule. The existence of a *m/e* 166 (ee) peak points toward their location in different rings and the substantial loss of water from M − 43 (ff, *m/e* 245), accompanied by a metastable ion at 228.2 ($245^2/263 = 228.2$), supports the assigned structure.

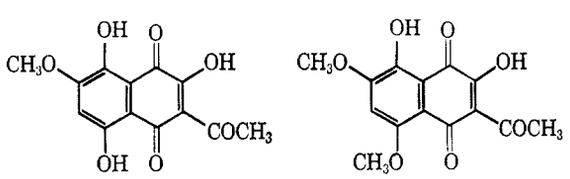


11

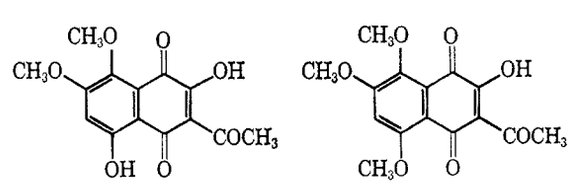


ee, *m/e* 166 ff, *m/e* 245

Acetylmethoxynaphthoquinones.—In the spectra of acetylmethoxynaphthoquinones the fragmentation pattern established for acetyl derivatives is superimposed upon features determined by the methoxy function. As a useful generalization it can be stated that the acetyl group exceeds the methoxy group in its ability to direct disintegration upon electron impact in this class of compounds. When both functions are present in different rings, the spectrum follows more or less the pathway outlined for the corresponding unmethylated acetylnaphthoquinone derivative and is shifted by 14 mass units to higher mass according to the number of methoxy groups incorporated. In addition, however, features characteristic of the methoxy group can be detected. Figures 10–13, reproducing the spectra of different methylation products of spinochrome A[18] (**10**, Figure 8), illustrate these observations.



12 13



14 15

The spectrum (Figure 10) of 3-acetyl-2-hydroxy-7-methoxynaphthazarin[18] (**12**) exhibits the pattern outlined for **10** (see Table I). Loss of water from M − 43,

(17) I. Singh, R. E. Moore, C. W. J. Chang, and P. J. Scheuer, *J. Am. Chem. Soc.*, **87**, 4023 (1965).

(18) Methylation patterns of polyhydroxynaphthoguinones will be the subject of a forthcoming publication by R. E. Moore, H. Singh, C. W. J. Chang, and P. J. Schewer.

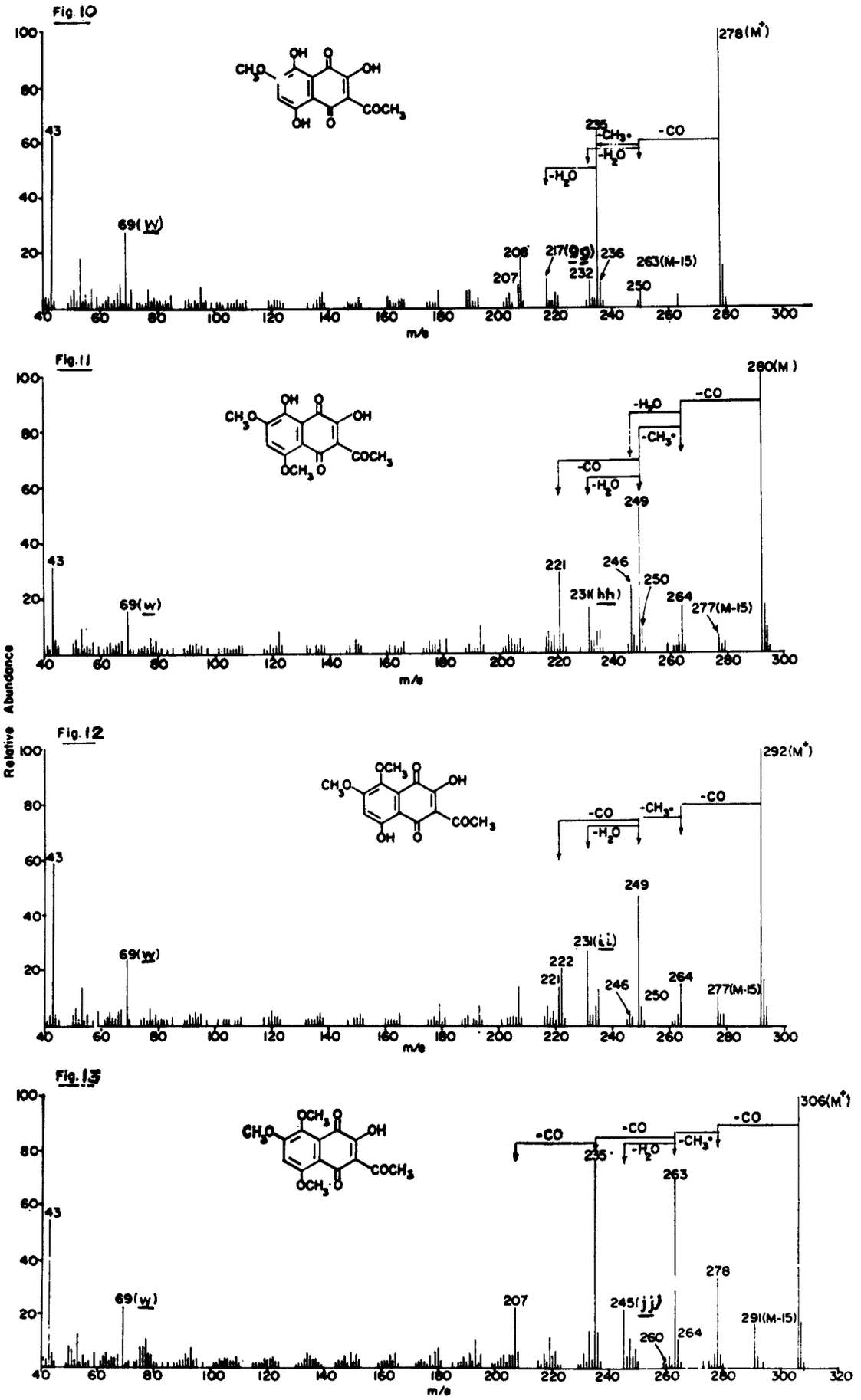


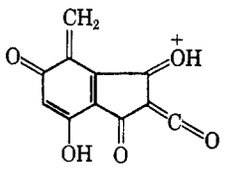
Figure 10.—Mass spectrum of 3-acetyl-2-hydroxy-7-methoxynaphthazarin.
Figure 11.—Mass spectrum of 2-acetyl-3-hydroxy-6,8-dimethoxyjuglone.
Figure 12.—Mass spectrum of 3-acetyl-2-hydroxy-7,8-dimethoxyjuglone.
Figure 13.—Mass spectrum of 3-acetyl-2-hydroxy-5,7,8-trimethoxynaphthazarin.

TABLE I

ANALOGOUS FRAGMENTS OF SPINOCHROME A AND ITS METHYL DERIVATIVES (INTENSITY IN PER CENT TOTAL IONIZATION, $\Sigma_{70}^{M^+}$)

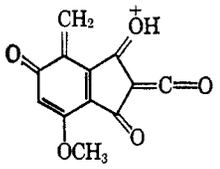
		M	M − 15	M − 28	M − 42	M − 43	M − 28 − H_2O	M − 43 − H_2O	M − 70	M − 43 − CO
					Spinochrome A					
10	*m/e*	264	249	236	222	221	218	203	194	193
	% TI	18.7	10.6	3.6	1.7	13.8	2.2	0.9	3.0	2.2
					Monomethylspinochrome A					
12	*m/e*	278	263	250	236	235	232	217	208	207
	% TI	15.1	0.6	0.8	1.4	9.5	1.4	1.5	2.7	1.4
					Dimethylspinochrome A					
13	*m/e*	292	277	264	250	249	246	231	222	221
	% TI	14.4	0.9	2.4	1.1	7.5	3.6	2.6	1.0	4.2
					Dimethylspinochrome A					
14	*m/e*	292	277	264	250	249	246	231	222	221
	% TI	14.1	1.3	2.1	1.0	6.8	0.4	4.0	3.0	2.0
					Trimethylspinochrome A					
15	*m/e*	306	291	278	264	263	260	245	236	235
	% TI	9.5	1.6	3.2	1.0	6.6	0.2	2.1	1.3	7.3
					d_3-Trimethylspinochrome A					
16	*m/e*	309	294	281	...	264	...	245	...	235
	% TI	4.9	0.9	2.4	...	3.6	...	0.8	...	4.2

amounting only to 0.9% of total ionization in the spectrum of **10** (Figure 8, *m/e* 203), is increased in intensity in its monoethyl ether up to 1.5%. The ion resulting from this dehydration may be depicted as gg (*m/e* 217).

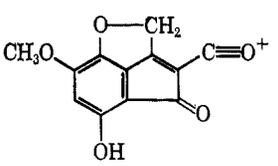


gg, *m/e* 217

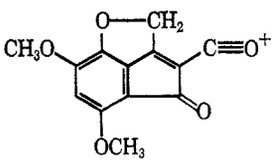
Two dimethylspinochromes A, mp 216–217° and 224–227°, have been obtained by further methylation of **12** and spectral studies suggest for these structures **13** and **14**, respectively.[18,19] The most remarkable difference between the mass spectra of the two isomers is the substantial loss of water from the M − 28 species in the spectrum (*m/e* 246 in Figure 11) of the 5-methylated derivative **13**, and its virtual absence in the case of the 8-methylated compound **14** (Figure 12). Trimethylspinochrome A[19] (**15**, Figure 13) again shows almost no elimination of water from M − 28, which gives rise to significant peaks in the spectra of **7** and **10**. Another ion of strongly varying intensity in the spectra of 3-acetyl-2-hydroxynaphthazarin derivatives is the peak at M − 70, corresponding to the loss of CO and ketene from the molecular ion. This fragment is outstanding for the 5-unmethylated compounds **7** (*m/e* 178, 15% relative intensity), **10** (cc, *m/e* 194), **11** (*m/e* 236), **12** (*m/e* 208), and **14** (*m/e* 222), but its contribution to total ionization is suppressed in the spectra of the 5-methylated derivatives **13** and **15** (see also Table I). Although no definite rationalization can be presented at this time for both fragmentation pathways, it seems that these features are related to the presence of the *peri*-hydroxyl group of the naphthazarin skeleton and that the hydroxyl at C-5 promotes the expulsion of ketene rather than water from the M − 28 ion whereas its absence enhances loss of water rather than ketene. Water can be lost from the M − (CO + CH_3) species in all three cases and the ions resulting from this fragmentation may be visualized in various forms of which hh, ii, and jj, respectively, are illustrative.



hh, *m/e* 231



ii, *m/e* 231



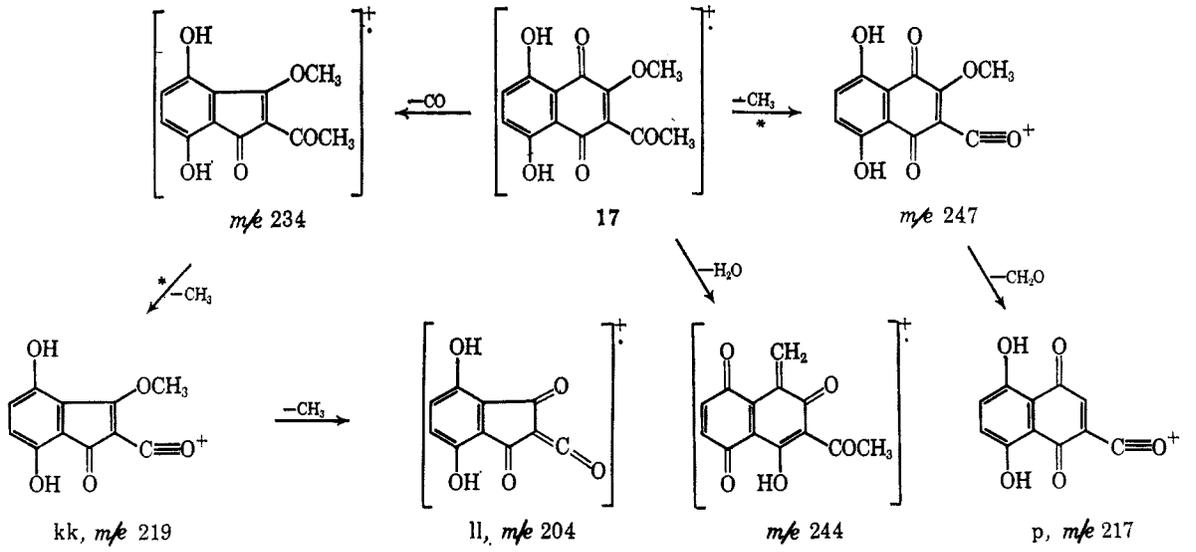
jj, *m/e* 245

The formulation of these ions, however, is based on the assumption, that the methyl group, which is lost to give rise to the M − (CO + CH_3) ion originates from the acetyl side chain. In order to prove this supposition, the acetyl hydrogens have been replaced by deuterium in **15** to give **16** (spectrum not shown). As expected (see Table I), the *m/e* 263 and 245 peaks remain unshifted in the spectrum of the deuterated compound. However, the M − 15 peak is moved three units to higher masses, thus showing elimination of a methyl radical from the molecular ion, as an event which occurs in the benzenoid part of the molecule. The increasing intensity of this peak going from the spectrum of **12** to that of **15** supports the labeling results.

In summary, it can be said, that apart from details characteristic for the structures of **12** and **15**, the major fragments of their spectra arise by the same pathway as outlined for spinochrome A (**10**), that is, initial expulsion of CO, followed by loss of a methyl from the acetyl group to form M − 43, which then breaks down further with elimination of CO and water. All four compounds **12–15**, on the other hand, have in common that the OH group in position 2 of the quinoid ring remains unmethylated.

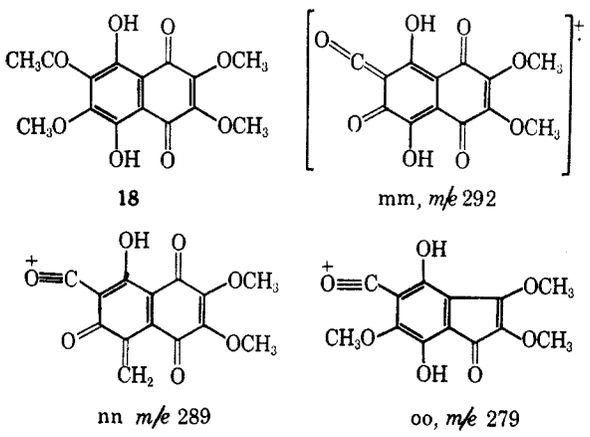
(19) Structure V of our preliminary communication[7] should be represented as **14** and the labels IV and V should be transposed.

SCHEME III



It is highly instructive, therefore, to note that methylation of the C-2 hydroxyl results in an inversion of the pattern just discussed. In the spectrum (Figure 14) of 3-acetyl-2-methoxynaphthazarin (**17**) (Scheme III) loss of methyl (*m/e* 247) from the molecular ion is favored over the initial expulsion of CO (*m/e* 234) and metastable ions at *m/e* 232.8 ($247^2/262 = 232.8$) and *m/e* 194.2 ($219^2/247 = 194.2$) indicate, that M − 43 (kk, *m/e* 219) can now arise from the M − 15 ion. The loss of a methyl radical from M − CO (*m/e* 234), however, still participates in the formation of kk, as is shown by an additional metastable ion at *m/e* 204.9 ($219^2/224 = 204.9$). A feature determined by the vicinal acetyl and methoxy functions in the benzenoid part of the molecule is the M − H_2O peak (*m/e* 244) probably forming in the same manner as for 2-methoxynaphthazarin.[20] The peak at *m/e* 232 corresponds either to the expulsion of formaldehyde from the molecular ion or, by analogy with the fragmentation pattern of 7-acetyl-2,3,6-trimethoxynaphthazarin (**18**, Figure 15), which is discussed below, is formed by loss of a second methyl radical from the M − 15 ion. Formaldehyde can be lost from M − 15 to give p (*m/e* 217). The ion of mass 204 (ll) is formed by loss of a second methyl group from kk, as indicated by a metastable ion at *m/e* 190.0 ($204^2/219 = 190.0$). Finally as might be expected, *m/e* 43 again constitutes an outstanding fragment.

Introduction of additional methoxy groups as in 7-acetyl-2,3,6-trimethoxynaphthazarin (**18**, Figure 15) has the result that fragmentation processes involving the methoxy groups dominate those characteristic for acetylnaphthoquinone. Thus loss of 15 mass units (*m/e* 307), characteristic for the location of the acetyl function in the benzenoid moiety, is followed by elimination of methyl[21] (mm, *m/e* 292), water (nn, *m/e* 289), CO (oo, *m/e* 279), and formaldehyde (*m/e* 277). Loss of water from the molecular ion yields *m/e* 304; expulsion of CO, on the other hand, does not occur to any noticeable extent. All transitions mentioned are supported by metastable ions.



Summary

The discussion of the spectra has shown, that the generalizations set out by Beynon[11] and Williams[10] and their co-workers are sufficient to rationalize the fragmentation patterns observed for naturally occurring hydroxy- and methoxynaphthoquinones.[22] For acetylnaphthoquinones, which constitute a large part of the Echinoderm pigment class, the following decomposition modes are operative and may be used for structure elucidation.

1. The acetyl function exceeds the hydroxy and methoxy groups in its ability to direct fragmentation of the molecule under electron impact.

2. 3-Unsubstituted 2-acetylnaphthoquinones can lose either the methyl group or ketene from the molecular ion. Subsequent expulsions of carbon monoxide and acetylene from both initial fragments lead to a highly characteristic "doublet" pattern.

3. In 3-acetyl-2-hydroxynaphthoquinones the hydrogen bonding between the hydroxy and the acetyl

(20) Water is lost from the molecular ion in the spectrum of 2-methoxynaphthazarin. Since this fragmentation is completely absent in the spectrum of 2-methoxynaphthoquinone, the *peri* hydroxyl is probably involved. In the spectrum of d_3-methoxynaphthazarin, the *m/e* 202 peak (M − 18) has shifted to *m/e* 204 thus indicating, that a methoxy proton is involved in this loss of water.

(21) Established by a high-resolution measurement of mm.

(22) See also S. J. DiMari, J. H. Supple, and H. Rapoport, *J. Am. Chem. Soc.*, **88**, 1226 (1966).

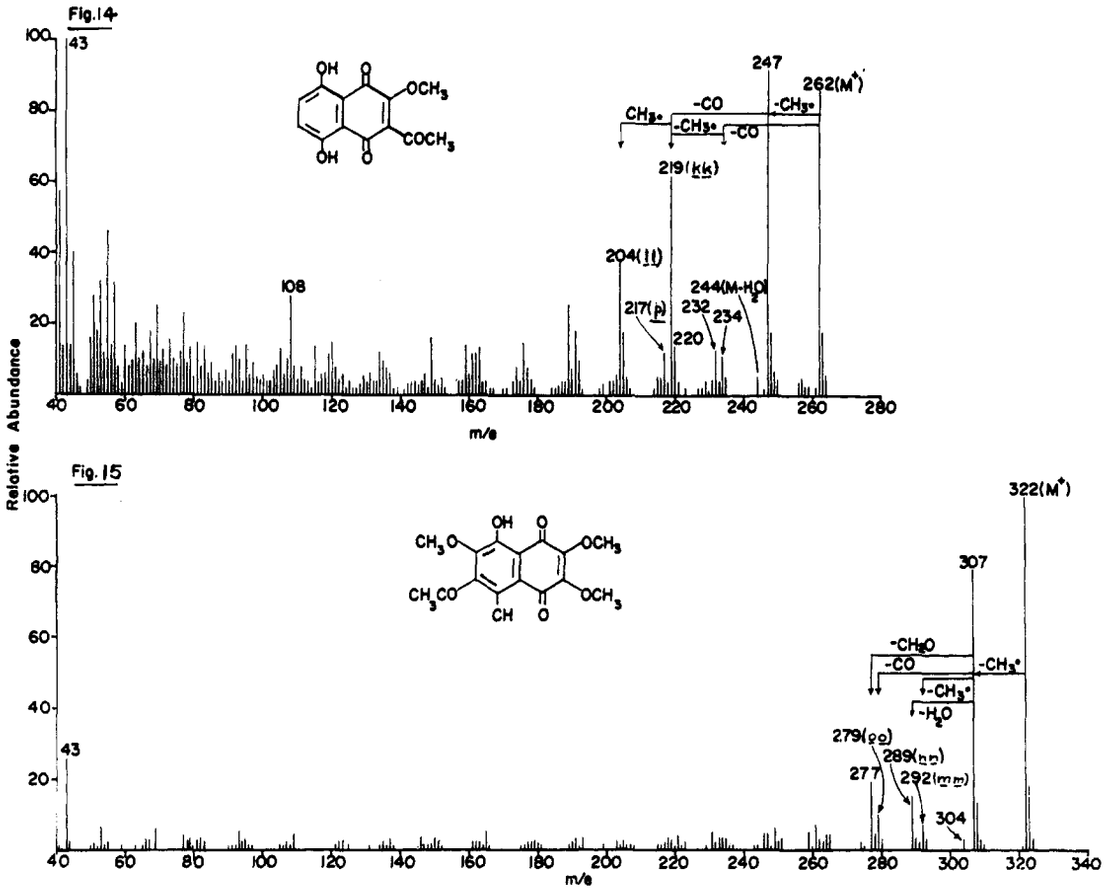


Figure 14.—Mass spectrum of 3-acetyl-2-methoxynaphthazarin.
Figure 15.—Mass spectrum of 7-acetyl-2,3,6-trimethoxynaphthazarin.

function favors the elimination of carbon monoxide as a first fragmentation step, which is followed by loss of a methyl radical to form a very stable M − 43 peak. In addition, water and/or ketene can be lost from the M − CO species.

4. If the acetyl function is located in the benzenoid moiety of the molecule, a methyl group is lost first, followed by the expulsion of carbon monoxide. Loss of ketene from the moleular ion can be noted, but is less pronounced than in the 3-unsubstituted 2-acetylnaphthoquinones.

5. Acetylmethoxynaphthoquinones exhibit in general the pattern expected for the unmethylated acetylnaphthoquinone derivative, if methoxy and acetyl functions are part of different rings. If both substituents are located in the quinoid moiety, the vicinity of the two groups determines the breakdown of the molecule under electron impact.

Thus, a group of empirical rules is now available for the interpretation of the mass spectra of naphthoquinone derivatives, which can be used successfully together with other physicochemical methods for structure elucidation in this class of compounds.

Experimental Section

The mass spectra were recorded with an AEI-MS 9 mass spectrometer operating with an ionization energy of 70 ev. The temperature of the ion source was 200°. Samples were introduced into the source using the direct inlet system. In order to check on the influence of instrumental conditions (heated inlet *vs.* direct inlet system), some of the spectra reported by Williams, *et al.*,[10] were rerun with virtually identical results.

Methoxynaphthazarin-d_3.—Diazomethane-d_2 was prepared from N-nitrosomethyl-d_3-urea. A solution of 2 mg of naphthopurpurin-d_3 in 3 ml of absolute deuterium methoxide was treated very carefully with a dilute solution of the diazomethane-d_2 in absolute deuterium methoxide. The hexadeuterio product was purified by chromatography on acid-treated deactivated silica gel. Mass spectral analysis showed 82% exchange of the methoxyl protons and also indicated the presence of a tetradeuterio species (*ca.* 3%) owing to the slow exchange of the C-3 proton before methylation.

2-Hydroxy-3-acetyl-d_3-5,7,8-trimethoxy-1,4-naphthoquinone (Trimethylspinochrome A-d_3).—Sodium (100 mg) was dissolved in 3 ml of absolute deuterium methoxide, 5 mg of trimethylspinochrome A was added, and the resulting solution was allowed to stand at room temperature for 3 hr. After acidification with phosphorus pentoxide in deuterium oxide, the product was extracted with ether and the ethereal layer was washed with water, dried, and evaporated. The nmr spectrum in deuteriochloroform revealed essentially complete exchange by the absence of the acetyl signal.