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THE MASS SPECTRA OF THE KYNURENINES

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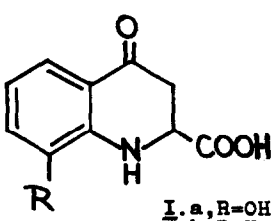
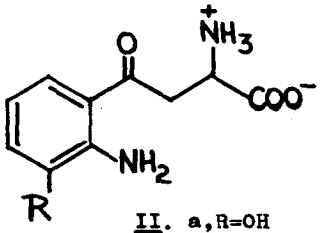
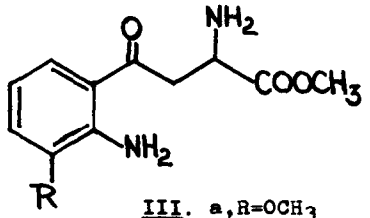
It has recently been established that the pigment isolated from butterfly wings (genus Heliconius and subfamily Ithomiinae) (1) is not L-hydroxykynurenine yellow (Ia) (2) as originally proposed, but 3-hydroxy-L-kynurenine (IIa) (3). We have investigated the mass spectra of the two compounds, as well as of the parent non-hydroxylated derivatives (Ib, IIb) and of the oily methylation products of the kynurenines, under identical conditions (MS-9, direct inlet system). The spectrum of the pigment (Fig. 1) showed no molecular ion (which was the primary cause of the original erroneous structural assignment), while that of kynurenine (IIb) showed only a very faint molecular ion (Fig. 2). The spectra of IIa-b were quite similar to those of the cyclized derivatives Ia-b (Figs. 3-4), the main differences being in the relative intensities of the peaks corresponding to M^+ or spurious M^+ less CO_2 or COOH (m/e 162-3, 146-7), peaks derived from m/e 163 and 147, and the observation of peaks from the kynurenines at m/e 136 and 120, and 120 and 104, respectively.



I. a, R=OH
b, R=H



II. a, R=OH
b, R=H



III. a, R=OCH3
b, R=H

In order to further understand the fragmentation pathway of the kynurenines, high-resolution measurements were made on major peaks of the spectra of the pigment and of kynurenine methyl ester IIIb (Fig. 6). It became evident that the kynurenines undergo cyclization with loss of ammonia either in the heated inlet system or upon electron impact; even the esters IIIa-b show no or very weak molecular ions under the conditions used.* The compounds can evidently also fragment without necessary cyclization, to give the characteristic peaks at m/e 136 ($C_7H_6NO_2$), 120, and 150, corresponding to cleavage adjacent to the keto group in II-III; these peaks are absent from the spectra of the cyclized materials I. Furthermore, the cyclized intermediate evidently can lose CO_2 before rearrangement to the molecular ion of I (which then fragments essentially as does I), to give the base peaks at m/e 163 and 147.

The failure of the methylated pigment IIIa (Fig. 5) to give peaks corresponding to loss of CO, and the reduced intensities of m/e 104, 117, 118, and 119 in the spectrum of kynurenine (Fig. 2), imply that the CO is lost partially but not wholly from the phenolic grouping, following a well-documented fragmentation (5). Loss of CH_3· from the m/e 135 (or 120) peak in IIa-b was documented by the observation of the appropriate metastable peak as well as high-resolution measurements. The strong peaks at m/e 161 and 133 in IIIa, not present in the spectrum of the demethoxy-analog IIIb, suggest a possible loss of methyl from the methoxyl group, a fragmentation previously supposed but not rigorously proven in many indole alkaloids (K.Brown, unpublished observations). Both of the esters (IIIa-b) show loss of carbomethoxyl before cyclization and loss of methoxyl as well as carbomethoxyl after, the formulae of the fragments being established by high-resolution measurements.

The probable fragmentation pathways for the kynurenines and their esters are outlined in Charts I and II, with the supporting spectra in Figs. 1-6;

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* This observation is supported by results of Biemann and co-workers, (4) who found that the base peak in the spectrum of lysine ethyl ester corresponds to the elimination of NH_3 from M - $COOC_2H_5$:

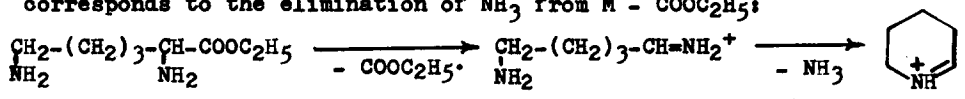


FIG. 1

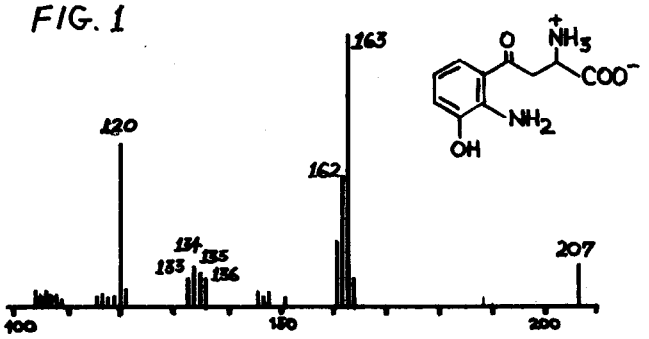


FIG. 2

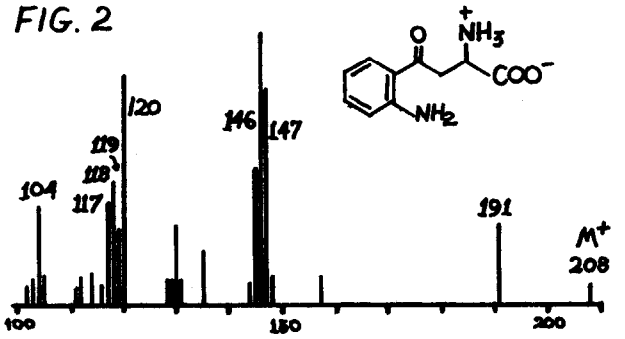


FIG. 3

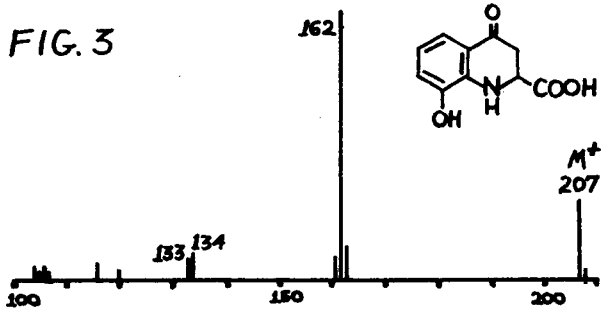


FIG. 4

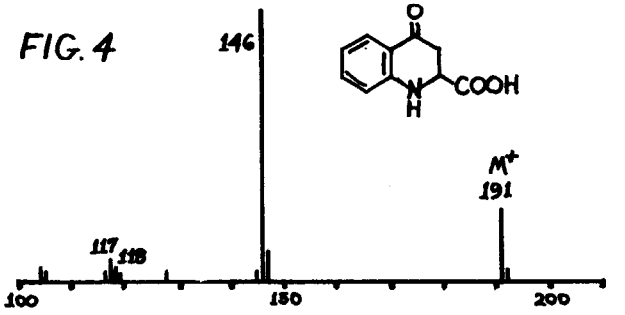


FIG. 5

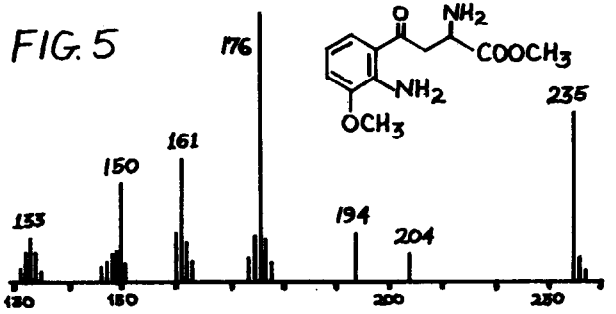


FIG. 6

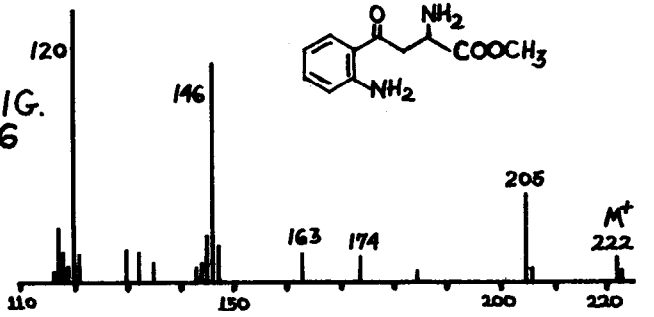


CHART I

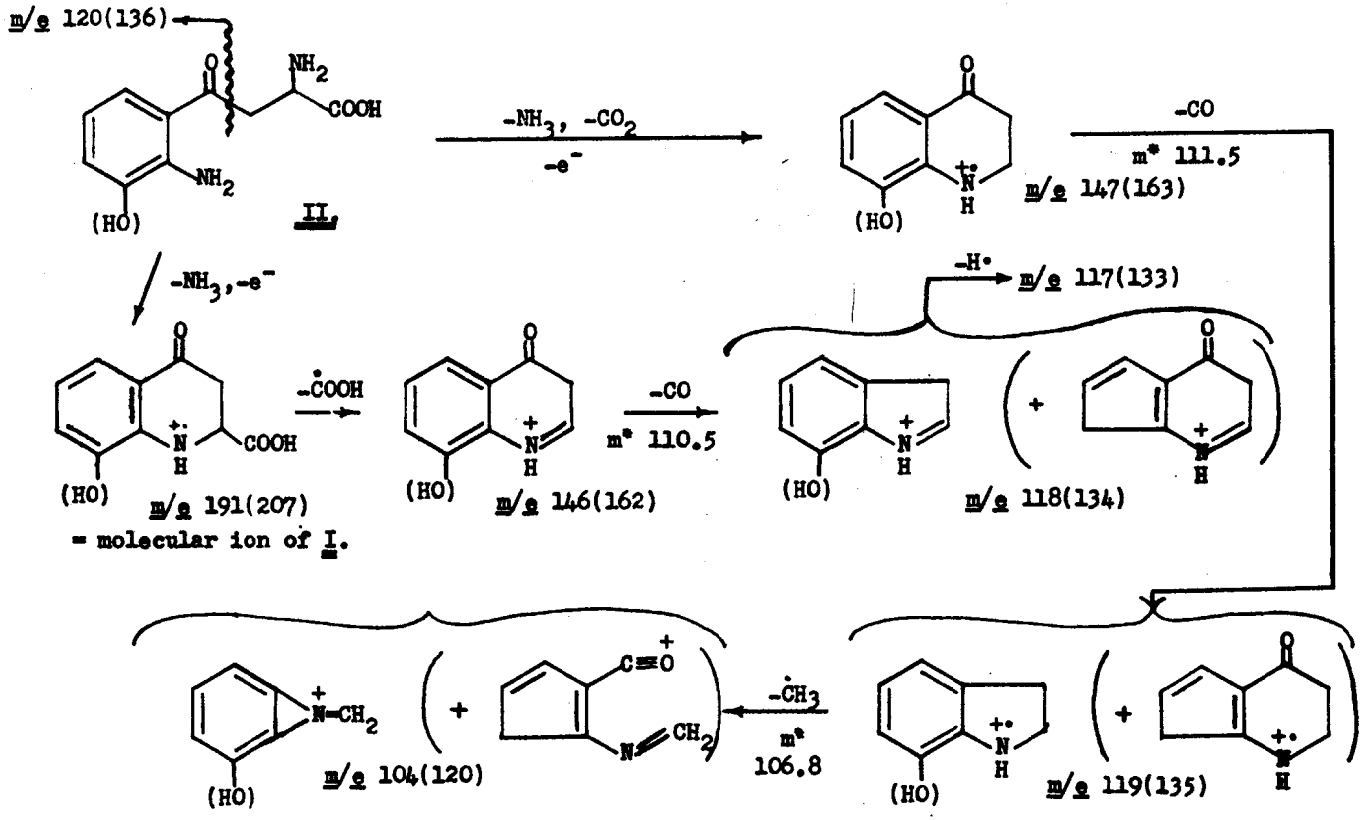
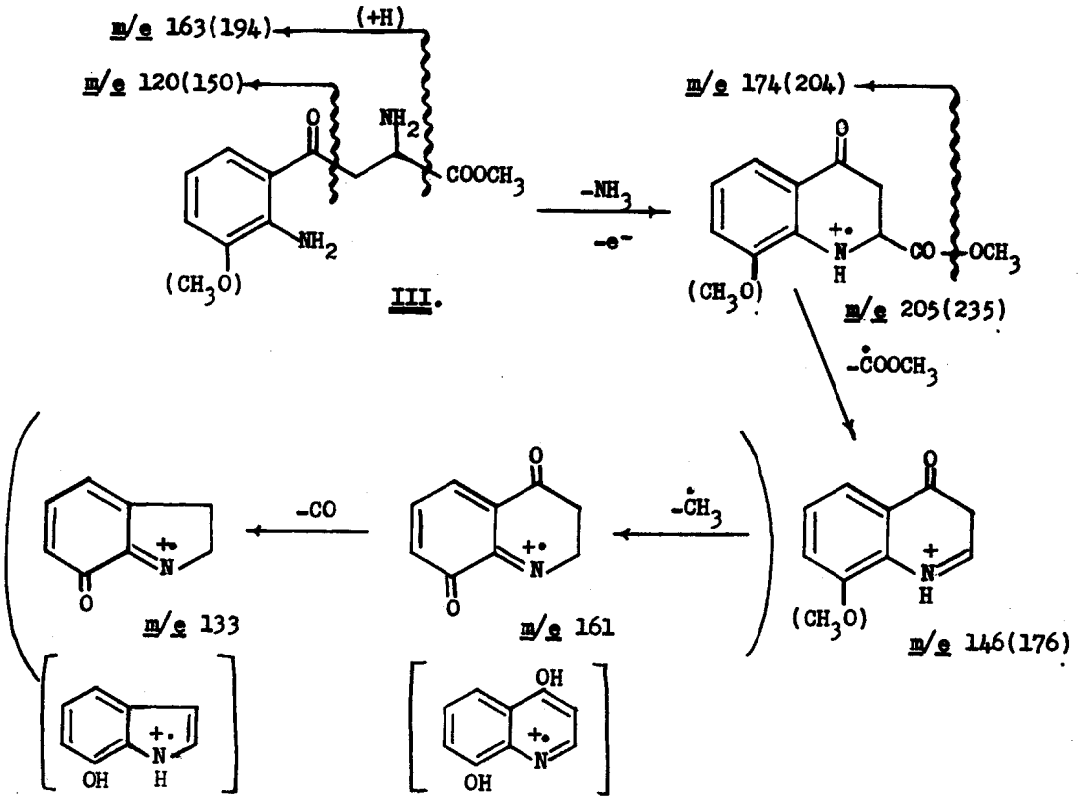

m/e 120(136)
II
-NH3, -CO2
-e-
-CO
m* 111.5
m/e 147(163)
-H•
m/e 117(133)
-NH3,-e-
-•COOH
-CO
m* 110.5
m/e 191(207)
= molecular ion of I.
m/e 146(162)
m/e 118(134)
-•CH3
m*
106.8
m/e 104(120)
m/e 119(135)

CHART II


m/e 163(194)
(+H)
m/e 120(150)
NH2
COOCH3
NH2
(CH3O)
III.
-NH3
-e-
m/e 174(204)
CO
OCH3
(CH3O)
m/e 205(235)
-·COOCH3
-·CH3
-CO
m/e 133
m/e 161
m/e 146(176)
OH
H
OH
OH

the high-resolution work is summarized in Table I, below.

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3. T. Tokuyama, S. Senoh, T. Sakan, K.S. Brown, Jr. and B. Witkop, J.Amer.Chem.Soc., in press (March 1967).

4. K. Biemann, Mass Spectrometry, pp. 265-6. McGraw-Hill, New York (1962).

5. See H. Budzikiewicz, C. Djerassi, and D.H. Williams, Interpretation of Mass Spectra of Organic Compounds, pp. 167-8. Holden-Day, San Francisco, Calif. (1964).

TABLE I: High-resolution Mass Spectral Measurements

Peak	Observed Mass	Expected Formula	Calculated Mass
Compound IIa (3-hydroxy-L-kynurenine)			
207	207.0541	$C_{10}H_9NO_4$	207.0532
163	163.0636	$C_9H_9NO_2$	163.0633
136	136.0397	$C_7H_6NO_2$	136.0399
135	135.0681	C_8H_9NO	135.0684
134	134.0604	C_8H_8NO	134.0606
133	133.0527	C_8H_7NO	133.0528
120	120.0447	C_7H_6NO	120.0449
Compound IIIb (DL-kynurenine methyl ester)			
174	174.0553	$C_{10}H_8NO_2$	174.0555
163	163.0870	$C_9H_{11}N_2O$	163.0868
120	120.0451	C_7H_6NO	120.0449