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The Excretion of Ethylquinone by the Flour Beetle

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While studying inert dust insecticides we observed that the red rust flour beetle (*Tribolium castaneum*) made a brown stain when kept in contact with various fine white powders such as amorphous precipitated silica. When placed on these dusts and kept in dry air the insects did not move about in the material after the first few hours and died after about 2 days. The brown stain developed uniformly round the insect and became visible after the first day, but did not reach its maximum strength until about the sixth day, i.e. long after the insect had died. A similar but smaller stain was produced when dead insects were placed on the powder.

The closely related insect, *Tribolium confusum*, is known to give off an excretion which causes heavily infested flour to become pink and quite unpalatable, and which Payne [1925] found to exhibit aldehyde reactions. Chapman [1926] observed that the same beetle, when stimulated, gave off a nose-irritant gas which turned flour pink. He examined the effect of this gas on the pupae of the beetle and found that the subsequently metamorphosed adults were abnormal. Roth & Howland [1941] passed dry air over *T. confusum*, and isolated the excretion as yellow-brown needles which turned red and eventually blue, even when kept in solid CO₂. At room temperature a gas was evolved of quinone-like odour which was capable of oxidizing HI to I₂. These authors found that the last instar larvae, prepupae and pupae of the insect all metamorphosed into abnormal adults after exposure to this gas.

EXPERIMENTAL

Isolation of the excretion from T. castaneum

The method used for isolating the excretion from *T. castaneum*, based on that used by Roth & Howland [1941] for *T. confusum*, was as follows: About 500–1000 insects (a

volume of about 5 ml.) were collected from the insect cultures by taking advantage of the negative geotropism of the adult beetles, sifted free from flour and introduced into a small distilling flask. A stream of dry air was then passed through the flask for about 6 hr., during which time the insects were cooled from 35–40 to 0° for about 20 min. every 2 hr. The excretion was condensed in an ethanol-CO₂ trap, yielding about 0.5 mg. of long, needle-like yellow crystals together with some ice, from the H₂O also given off by the insects. The yield was considerably reduced if the insects were not completely free from flour and larvae, etc. The insects were returned to flour for 3 days and then the procedure was repeated, giving a somewhat smaller yield. After three or four such operations the insects were very feeble and yielded little or no excretion. Many were dead, due, probably, to desiccation. Attempts to separate the H₂O from the excretion by drying with P₂O₅, CaCl₂, Na₂SO₄ or Mg(ClO₄)₂ yielded a reddish 'polymer' of the excretion on the surface of the drying agent, even when the drying tubes were warmed to 40°.

In view of the small yield per insect (about 0.1–0.2% of the initial body weight) the excretion from some 5000 beetles was necessary for the investigation outlined below.

RESULTS

Properties of the excretion from T. castaneum

The yellow crystals, separated from the H₂O on a cooled porous tile, had a nose-irritant quinone-like odour and were slightly lachrymatory. They were slightly soluble in H₂O, but readily so in ethanol, benzene, chloroform and other organic solvents. The substance was fairly volatile at room temperature and rather labile when first isolated, turning to a red-brown on standing overnight, even at 0°. However, drying increased the stability. The aqueous solution was neutral, reduced Tollen's reagent, liberated I₂ from HI, and gave a brown-black colour with warm dilute alkali. The substance was very sensitive to alkali, but comparatively stable to acid.

No colour was produced with conc. HCl or with FeCl₃. When SO₂ was passed through the aqueous solution the yellow colour was discharged and a black precipitate was formed, which later dissolved. N, S, P, and halogens were absent, and tests for aldehyde, ketone, alcohol and similar groups were negative. This chemical evidence identified the compound as a quinone. The m.p. of the dry crystals was 23°, but when the dry substance was allowed to stand at room temperature for several hours the m.p. rose to about 32°.

Preparation and properties of the hydroquinone. The excretion product was reduced immediately after isolation by a solution of NaHSO₃ sat. with SO₂. This solution was extracted three times with ether and the combined ether extracts were washed with NaHCO₃ and then with H₂O. The ether-soluble fraction was dried azeotropically with benzene, and recrystallized from benzene-ligroin mixture. In this way about 8 mg. of white crystals were obtained, melting at 97–98°. Analysis (Mr W. F. Boston) showed C=68.8; H=7.0% (cf. toluhydroquinone C, 67.7; H, 6.45%; ethylhydroquinone C, 69.5; H, 7.2%). A further quantity of this hydroquinone, prepared from an additional supply of insects, melted at 101–102° after 4 crystallizations from benzene-ligroin mixture, but there was insufficient material to continue the purification.

An aqueous solution of the hydroquinone was titrated electrometrically with N/25 Ce(SO₄)₂ (standardized by a similar titration against pure hydroquinone). Three determinations were carried out on about 1.5 mg. with an error of less than 1%. The oxidation equivalent weight thus determined was 67. (Theoretical values: toluhydroquinone 62; ethylhydroquinone 69.) Pure ethylhydroquinone gave an equivalent of 70 when titrated in this way.

The m.p.'s, however, of both the insect quinone itself, and of the hydroquinone produced therefrom, differ considerably from those recorded for pure ethylquinone and ethylhydroquinone, but these did not give depressions when admixed with authentic ethylquinone and ethylhydroquinone respectively, whereas there were marked depressions with other homologous members of the series, prepared by standard methods [Johnson & Hodge, 1913; Gulland, 1932; Cook, Heilbron & Lewis, 1942].

Properties of the excretion from T. confusum

A similar examination has been made of the excretion of *T. confusum*. This seems also mainly to be ethylquinone. The yellow crystals obtained had the same m.p. and other properties as the excretion from *T. castaneum*. No volatile excretion could be isolated in the same way from the meal worm beetle (*Tenebrio molitor*) or from the saw-toothed grain beetle (*Oryzaephilus Surinamensis*).

The ultra-violet absorption spectra of some quinones and hydroquinones

While many *p*-benzoquinones have been isolated from natural sources in recent years, descriptions of their ultra-violet absorption spectra are scanty. The naphthaquinones [Morton & Earlam, 1941] have been more fully covered.

Previous determinations of the value of $\lambda_{\max}$ for *p*-benzoquinone itself have in general been in good agreement with each other, but there is a confusion as to the value for toluquinone in ethanol [cf. Lifschitz, Lourie, Zimmermann & Huinick, 1924; Light, 1926]. This confusion probably results from the labile character of mono-substituted alkylquinones in hydroxylic solvents. Thus, the absorption spectrum of toluquinone in ethanol rapidly changes (Table 1) on standing for only a day at room temperature in diffuse daylight.

Table 1. *Ultra-violet absorption spectrum of toluquinone in ethanol*

Time after making up solution	$\lambda_{\max}$, m μ	$\epsilon_{\max}$.
ca. 1 min.	245	17,600
1 day	256	8,300
7 days	265	6,350
	228	3,650
	(inflection) 290	2,000

The data in Table 2 were therefore obtained immediately after the solution in ethanol of the freshly sublimed quinone had been made up.

Table 2. *Ultra-violet absorption spectra of freshly prepared quinones*

Quinone	Solvent	$\lambda_{\max}$, m μ	$\epsilon_{\max}$.
Benzo-	Ethanol	242	13,800
Tolu-	Ethanol	245	16,000
Ethyl-	Ethanol	246	16,500
Ethyl-	<i>n</i> -Hexane	246	14,800
Thymo-	Ethanol	255	20,600
Insect-	Ethanol	245	—

$\lambda_{\max}$ increases with increasing substitution of the quinone chromophore, an observation in good agreement with previous experimental results with analogous chromophores [cf. Woodward, 1941; Gillam, Barker & Evans, 1940; Gillam & Evans, 1941]. The spectrum of the insect quinone ($\lambda_{\max} = 245\text{m}\mu$) is that of a mono-substituted benzoquinone. This was confirmed by a comparison (Table 3) of the absorption spectrum of the hydroquinone prepared from the insect quinone with the absorption spectra of a number of mono-substituted hydroquinones. All determinations were carried out in ethanol.

Table 3. Absorption spectra of hydroquinones in ethanol

	$\lambda_{\max}$, m μ		$\lambda_{\min}$, m μ		λ_{inflex} , m μ	Remarks
	I	II	I	II		
Hydroquinone						
Tolu-	228	293	224	251	None	Two maxima
Ethyl-	None	292	None	ca. 250	ca. 231	—
n-Propyl-	None	295	None	ca. 253	ca. 228	Shift of maximum to longer wave-length
Insect-	None	292	None	252	ca. 228	—
	$\epsilon_{\max}$		$\epsilon_{\min}$		ϵ_{inflex}	Ratio E ca. 290 m μ to E at ca. 220 m μ
	I	II	I	II		
Hydroquinone						
Tolu-	4750	3950	4500	800	None	0.83
Ethyl-	None	3500	None	—	3850	0.74
n-Propyl-	None	3400	None	—	4800	0.71
Insect-	None	—	None	—	—	0.69

DISCUSSION

The evidence leaves little doubt but that the main part of the *Tribolium* excretion is ethylquinone. The other material present in the excretion is probably more volatile than ethylquinone (since the m.p. of the dry insect excretion rises on standing in the air without apparently 'polymerizing'), has a lower C content and therefore a higher O content, has a lower equivalent with respect to $\text{Ce}(\text{SO}_4)_2$ oxidation and is probably reduced by SO_2 ; hence it cannot be a simple *p*-benzoquinone derivative.

The excretion of ethylquinone by *Tribolium* is undoubtedly the cause of the pink colour of 'conditioned' flour, the production of the colour being due to the reaction of simple *p*-benzoquinones with amino compounds such as the protein in flour [cf. Gulland, 1932; Fujita & Kodama, 1935; Cooper & Nicholas, 1927]. 5 mg. of toluquinone added to 10 g. of fresh flour rapidly causes the latter to turn pink and to take on the appearance of 'conditioned' flour. Ethylquinone and other simple quinones also cause stains, when placed on amorphous precipitated silica, similar to those caused by the insects themselves. The stain is apparently due to the 'polymerization' of the quinone [cf. Erdtman, 1933].

Apart from the complex anthraquinone derivatives obtained from *Coccus laccae*, *C. ilicis* and *C. cacti* [see Todd, 1941], ethylquinone appears to

be the first *p*-quinone to be isolated from an insect source. This is surprising in view of the abundance of *p*-quinones as metabolic products of fungi.

No definite function can at present be ascribed to the ethylquinone excreted by *Tribolium castaneum*, but it is perhaps significant that ethylquinone is a strong spermicide, at least for guinea-pig sperms [Gulland, 1932], and it may well play a part in the remarkable population control in crowded cultures of *Tribolium* [e.g. Park, 1936; Crombie, 1942].

SUMMARY

1. The main part of the volatile excretion of *Tribolium castaneum* has been identified as ethylquinone. The same substance was obtained from *T. confusum*. While, owing to the very small amount of material available, pure ethylquinone could not be isolated, the evidence collected appeared conclusive.

2. The ultra-violet absorption spectra of a number of alkyl-substituted *p*-quinones and hydroquinones are recorded.

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