Spontaneous Deoxygenation of N-(3,4-Methylenedioxybenzyl)-Acetohydroxamic Acid: An Exceptionally Facile Process

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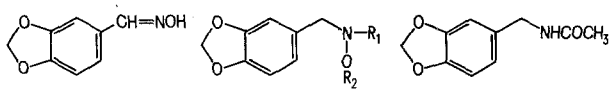
Summary. An exceptionally facile spontaneous deoxygenation of N-(3,4-methylenedioxybenzyl)-acetohydroxamic acid to its corresponding amide, without apparent literature precedent, is reported.

In the course of devising a new reductive rearrangement[2] for the synthesis of biologically important peptide derivatives, we had occasion to prepare N-(3,4-methylenedioxybenzyl)acetohydroxamic acid **4** and present here evidence for its unusually rapid thermal decomposition to the corresponding acetamide. In order to obtain an authentic sample of **4**, standard procedures were employed



1 **2** $R_1 = R_2 = H$; **3** $R_1 = R_2 = COCH_3$; **4** $R_1 = COCH_3$, $R_2 = H$; **5**

as outlined in Scheme I. Reduction of **1** (m.p. 110°) using diborane[3] afforded the hydroxylamine **2**, m.p. 43–45° [NMR ($CDCl_3$) δ 3.92 (s, 2H), 5.60 (broad s, 2H; NH, OH), 5.98 (s, 2H), 6.8 (narrow m, 3H); IR ($CHCl_3$) λ_{max} 2.9, 6.6, 6.9 μ; mass spectrum (C.I.) 167 (M^+), 135 (base, $C_8H_7O_2^+$)]. Exhaustive acetylation of **2** (Ac_2O) furnished the oily N,*O*-diacetyl derivative **3** [NMR ($CDCl_3$) 2.03 (s, 3H), 2.14 (s, 3H), 4.75 (s, 2H), 5.95 (s, 2H), 6.76 (narrow m, 3H); IR (film) 5.58, 6.0 μ]. This substance underwent rapid hydrolysis (0°, 30 min) in dilute aqueous NaOH to a monoacetyl derivative [NMR ($CDCl_3$) 1.98 (s, 3H), 4.2 (broad s, 2H), 5.92 (s, 2H), 6.76 (narrow m, 3H; underlying broad s, 1H); IR ($CHCl_3$) 3.0, 6.05 μ; mass spectrum 193 (M-16, base)] whose strongly positive reaction with $FeCl_3$ solution (deep burgundy in MeOH, EtOH) was characteristic of the hydroxamic acid **4**. Efforts to isolate **4** pure were unsuccessful. Whereas the substance appeared to be relatively stable at 0° in $CHCl_3$ solution (half-life ca. 2 days), efforts at chromatography (Florisil) or recrystallization yielded only the acetamide **5**, m.p. 104–105° (lit.[4] 103°) whose NMR-, IR-, and mass spectra matched perfectly those of an unambiguously prepared sample from piperonylamine and acetic anhydride.

Hydroxamic acids can be reduced to amides or lactams by a number of reagents[5]; however this remarkably facile deoxygenation of **4** is, to the best of our knowledge, unprecedented in the literature on hydroxamates.

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Species-Specific Protein Patterns in *Drosophila* Paragonial Glands[1]

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Summary. Electrophoretic analysis of the soluble proteins in the paragonial glands of 11 *Drosophila* species demonstrated that the patterns are highly species-specific. The possible functional significance of these proteins is discussed.

The paragonial glands (accessory glands) in the male flies of *Drosophila* have been studied by a number of investigators, mainly because of their specific roles in the reproductive process (see more recent reviews by CHEN[2] and FOWLER[3]). Extensive experiments carried out in our laboratory demonstrated that the secretory contents of these glands in *D. melanogaster*[4–6] and *D. funebris*[7–10] contain specific polypeptides and related amino acid derivatives which are responsible for the stimulation of oviposition and the reduction of receptivity in the mated females. We have also observed at least 12 electrophoretically separable proteins in the paragonial gland of *D. melanogaster*[11,12]. A polymorphism of one major protein band, which is autosomal in origin, could be detected. Recently, in a survey of the paragonial secretions in a total of 11 *Drosophila* species we found that the protein patterns are highly species-specific. This result will be dealt with in the present report.

Materials and methods. The flies of all *Drosophila* species were raised on a standard maize-agar-sugar-yeast diet at 25 °C. The males were separated from the females shortly after eclosion from the pupae and kept under the same condition with the diet renewed every 4 days. The classification of the 11 species included in this study is as follows: 1. subgenus *Sophophora* – *D. melanogaster*, *D.*

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simulans, *D. ananassae* (*melanogaster* group), *D. subobscura* (*obscura* group); 2. subgenus *Drosophila* – *D. virilis*, *D. americana* (*virilis* group), *D. nigromelanica* (*melanica* group), *D. funebris*, *D. macrospina*, *D. subfunebris* (*funebris* group); 3. subgenus *Dorsilopha* – *D. buscki*i[13,14].

For the preparation of protein extracts 20–50 pairs of paragonial glands were dissected from male flies of the desired age in a drop of ice-cold Ringer's solution. These were then homogenized in 20–40 µl Ringer's solution in an all-glass microhomogenizer at 4 °C. Following centrifugation at 7,000 rpm for 15 min at 4 °C, the clear supernatant fraction was employed for electrophoresis.

The proteins were separated by polyacrylamide gel electrophoresis according to the method of DAVIS[15] as modified by BORNER[16]. The gel system consisted of a 2.5% sample gel (pH 6.7, 2 cm), a 3.5% spacer gel (pH 7.5, 1 cm) and a 7.5% separation gel (pH 8.9, 7 cm). The electrophoretic run was carried out at 4 °C with a constant current of 0.5 mA per tube for the first 30 min and 1 mA per tube for another $1^1/_2$ h. The protein bands were then stained with 1% amido black and washed in 7% acetic acid.

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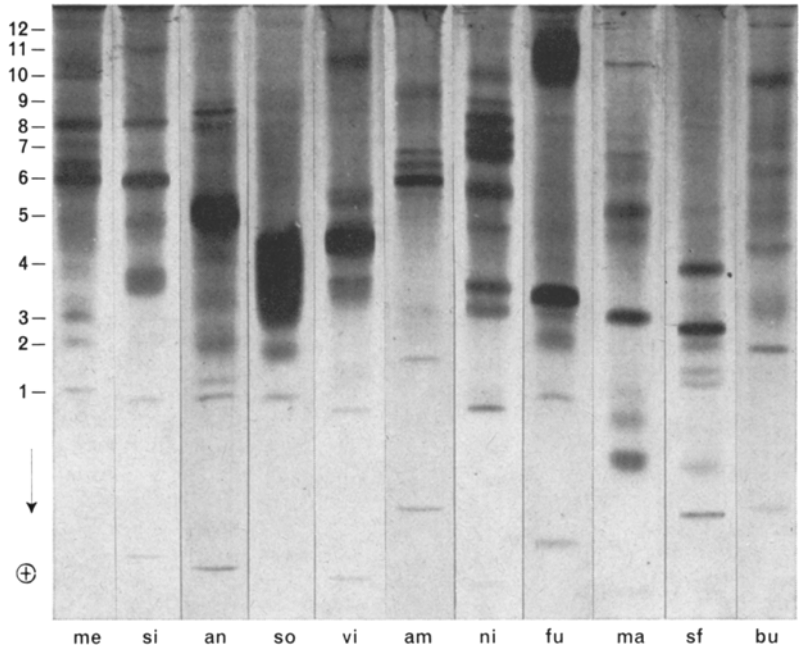

Fig. 1. Electropherograms of paragonial proteins from adult males of *D. melanogaster* (me), *D. simulans* (si), *D. ananassae* (an), *D. subobscura* (so), *D. virilis* (vi), *D. americana* (am), *D. nigromelanica* (ni), *D. funebris* (fu), *D. macrospina* (ma), *D. subfunebris* (sf) and *D. buscki*i (bu).

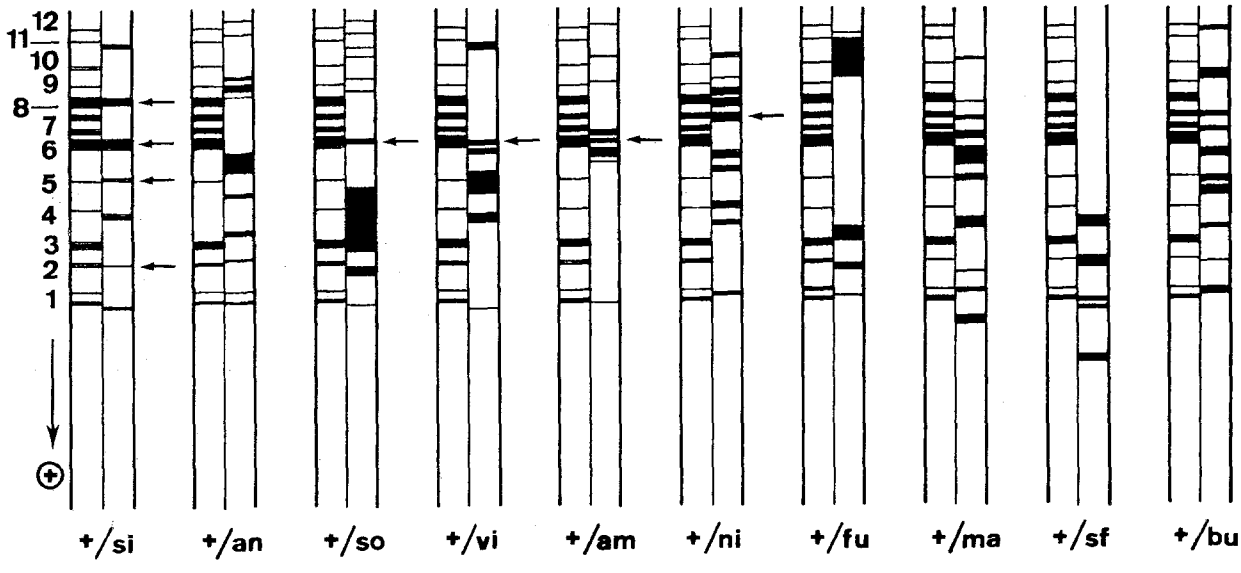

Fig. 2. Comparison of the electrophoretic mobilities of paragonial proteins between *D. melanogaster* and other *Drosophila* species by the split-gel technique. In each diagram the left half represents the protein pattern of *D. melanogaster* (+) and the right half that of the species to be compared. Abbreviations of the different species are given in the legend of Figure 1. The horizontal arrows indicate protein bands of identical mobilities on each gel.

In order to compare the electrophoretic mobilities of the protein fractions from different species on the same gel, the split-gel technique was used[17]. A small piece of cellulose acetate foil was inserted into the upper surface of the sample gel to prevent the mixing of protein samples applied to the same gel. The electrophoretic conditions were otherwise the same as stated above.

Results and discussion. As illustrated by the electropherograms in Figure 1, the protein patterns of paragonial proteins in the different species differ greatly in both the numbers and concentrations of individual bands. Striking differences in the anodal mobilities of the major bands are particularly evident. The age of the flies employed for analysis varied from 10 to 23 days post emergence. There is a progressive accumulation of secretion in the paragonial glands when the males are prevented from mating. However, our experience showed that this accumulation has no effect on the protein pattern. We estimated that in the paragonial glands of flies aged 10–15 days the secretory proteins account for approximately $^3/_4$ of the total soluble protein[18]. This means that the electrophoretic patterns shown in Figure 1 represent mostly the secretory proteins, including the needle-like 'crystals'[19] and the 'filamentous bodies'[20,21] secreted by the glandular cells. By using 10% SDS-gels VON WYL[12] estimated that the paragonial proteins in *D. melanogaster* have molecular weights from 12,000 to 120,000. Although corresponding data for the other species are not available, they are probably within the same range.

In order to determine the electrophoretic mobilities under more comparable conditions we employed the split-gel method by using the protein pattern of *D. melanogaster* as a standard. In each electrophoretic run, the paragonial extract from one species and that from *D. melanogaster* were absorbed on to the same gel without, however, the two samples being mixed. Since in this procedure only half as much protein could be applied to the gel, the comparison had to be limited to the major bands. The results are summarized in the diagram in Figure 2. As can be seen, the elctrophoretic pattern of *D. simulans* shows the greatest similarity to that of *D. melanogaster*. From a total of 7 bands of *D. simulans* detected on the split-gel 4 have identical mobilities as bands 2, 5, 6 and 8 in *D. melanogaster*. On the other hand, not a single band in *D. ananassae* shows such an identity, though this species, as *D. simulans* and *D. melanogaster*, also belongs to the *melanogaster* group of the subgenus *Sophophora*. Among the remaining species only *D. subobscura, virilis, americana* and *nigromelanica* each exhibit one band with the same anodal migration as band 6 or 7 in *D. melanogaster*. From these results it may be concluded that there is no correlation between the similarity of the paragonial protein patterns and the taxonomic relationships of the *Drosophila* species examined in this study. Admittedly, electrophoretic mobility itself is no proof for the identity of the proteins. For a definite conclusion more crucial evidence from two-dimensional gel electrophoresis or immunoelectrophoresis is needed.

With regard to the functions of the paragonial proteins no definite information is available. Many suggestions have been made, without, however, convincing experimental support. The secretions of the paragonial glands are considered to play a role in the transfer, storage and utilization of the sperm (references in FOWLER[3]). They may also influence the behavior and reproductive physiology of the female following copulation. Transplantation of the paragonial gland or injection of its secretion into virgin females resulted in a distinct increase in egg deposition[22]. A similar response of the host females following transplantation of these glands from different species has also been reported[23]. The stimulation of such heterologous transplants is, however, difficult to be understood in view of the high species-specificity of the protein patterns, assuming, of course, that some protein component(s) in the paragonial secretion serves indeed as a trigger to stimulate fecundity. A meaningful discussion about the functions of the paragonial proteins must await future experiments with purified preparations of individual proteins.

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Effect of Cycloheximide on Germination-Induced Isocitritase Development and Decline in Intact and Excised Flax Cotyledons

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Summary. Removal of embryo axis before germination markedly prevents the development as well as subsequent decline in Flax cotyledon isocitritase. Cycloheximide inhibited the development and decline of the enzyme in intact cotyledons but prevented only the former in excised cotyledons.

It is well known that many events that occur in the cotyledons of germinating seeds are triggered and controlled by embryo axis. Numerous cotyledonary enzyme activities either fail to develop or develop only partially if the embryo axis is excized early during germination, and one or more auxins or cytokinins usually substitute for the axis[2,3]. Maximal development of isocitritase in squash cotyledons also requires the presence of embryo axis and benzyladenine replaces in part this requirement[4]. However, despite the strong evidence indicating the importance of both synthesis and degradation in maintenance of cellular enzyme levels in animal[5] and plant systems[6], no attempts appear to have been made to investigate the role of embryo axis, or substances

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