Biological Origin and Configuration of 10-Hydroxy-Δ²-decenoic Acid

We have recently established[1] that 10-hydroxy-Δ²-decenoic acid, which constitutes about 15 per cent of royal jelly, is not present in the free state in pollens of representative species, nectar or honey. We suggested that it might therefore be present in combined form or be a specific bee product. In order to investigate the latter possibility we have examined the four pairs of salivary glands of the honey bee (*Apis mellifica*) obtained by dissection under distilled water.

Alcohol suspensions of one hundred thoracic, post-cerebral, and hypopharyngeal glands and fifty mandibular glands obtained from worker bees were filtered, the residual glands extracted with ether (1 ml.) in a Mickel tissue disintegrator and the combined alcohol and ether extracts concentrated *in vacuo* to 0·2 ml. Each solution was analysed (50 μl.) by paper chromatographic separation in amyl alcohol/5 *M* formic acid[2]. On spraying with a 0·1 per cent alcohol solution of chlorophenol red, an acidic component having R_F 0·88 identical with that of 10-hydroxy-Δ²-decenoic acid was detected only in the extract of the mandibular glands. A paper ionophoretogram[3] of the extracts run in 0·1 *M* borate buffer, *p*H 10·0 was dried, suspended in an atmosphere of formic acid vapour in a vacuum desiccator for 1 hr., the excess acid then allowed to evaporate and the residual acidic components detected with the indicator spray. A component having M_G value[3] 0·64 identical with that of 10-hydroxy-Δ²-decenoic acid was again found to be present only in the extract of mandibular glands. This was also confirmed by paper ionophoresis using 0·29 *M* acetate buffer, *p*H 5·0 and detection with alkaline silver nitrate[4].

The finding of 10-hydroxy-Δ²-decenoic acid in the mandibular glands of foraging bees is of interest; hitherto the hypopharyngeal glands have been considered the sole glandular source of larval food[5] although Haydak[6] noted that their contents assumed the appearance of royal jelly when treated with mandibular gland secretion. Whether a female larva develops into a queen or worker bee is determined by the nature of its food[7,8], and it has been suggested that the difference in diet occurs mainly with the older larvæ[7]. Two samples of larval food have therefore been analysed. The food from larvæ less than 3 days old was obtained by direct pipetting and that from older larvæ by filling the cells with distilled water and collecting the mixture after the larvæ had floated up to the top. Qualitative paper chromatographic and ionophoretic analysis of ether extracts of the larval foods indicated that the food from the larvæ less than 3 days old was richer in 10-hydroxy-Δ²-decenoic acid than that from the older larvæ.

The application of nuclear magnetic resonance spectroscopy has permitted the allocation of the *trans*-configuration to 10-hydroxy-Δ²-decenoic acid. The spectra were determined by one of us (L. M. J.) on a 10 per cent solution of methyl 10-hydroxy-Δ²-decenoate (obtained from the parent acid by diazomethane treatment) in carbon tetrachloride with Me_4Si as internal standard. It showed absorptions at (1) $\tau = 8{\cdot}65$ due to ordinary methylene protons[9], (2) $\tau \sim 6{\cdot}3$ due to methylene and methyl protons in $-CH_2OH$ and $-CO_2Me$ and (3) $\tau = 4{\cdot}2 - 2{\cdot}5$ associated with olefinic protons and typical of the AB region of an ABX_2 pattern where A and B are olefinic protons and X_2 the adjacent methylene group. The values $\tau_A = 3{\cdot}02$, $\tau_B = 4{\cdot}18$ p.p.m. and J_{AB} 15·8 c.p.s. were found. The coupling constant J_{AB} is correct for a *trans*- disubstituted ethylene (*cf.*, $J_{AB} \sim 12$ c.p.s. for *cis*- compounds) and the position of the β-proton ($\tau_A = 3{\cdot}02$) is close to that expected for a *trans*-compound (cf., $\tau_A = 2{\cdot}92$ for methyl *trans*-crotonate); the τ values for β-protons are critically dependent on stereochemistry[10].

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BIOCHEMISTRY

Application of Warburg's Equation to Tissue Slices

The possibility of using Warburg's equation to measure the diffusion coefficient of oxygen through slices of liver cut with the MacIlwain tissue slicer has been discussed[1].

Warburg's equation related the oxygen concentration outside to that at various points within a slice. The diffusion coefficient of oxygen through liver and the oxygen uptake by liver are also parameters in this equation. The equation is only valid when the respiration rate is independent of oxygen concentration. This is true above the critical oxygen concentration. Since cells furthest from the surface will respire at their maximal rate only when the oxygen concentration outside the slice is such that they are exposed to at least their critical oxygen concentration, it should be possible, knowing the critical oxygen concentration for the slice and for individual cells, the Q_{O_2} and the thickness of the slice, to calculate the diffusion coefficient.

For this application the equation reduces to:

$$C_c = C_s - \frac{ax^2}{2D}$$

where C_c = critical pO_2 (in atmospheres) of cells or mitochondria[2]; C_s = critical pO_2 (in atmospheres) of slice; a = ml. of oxygen consumed/min./ml. of tissue; $2x$ = slice thickness in cm.; D = diffusion coefficient.