85. *The Chemistry of Insects. Part I. The Wax of the Felted Beech Coccus.*

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The insect *Cryptococcus fagi*, Barensprung, lives on the trunks and larger branches of beech trees, which it often ultimately kills, and is to be reckoned among the more serious of the British insect pests. It secretes a protective felted mass of wax fibres, from which its

popular name, the felted beech coccus, is derived. A knowledge of the nature of this wax not only is of interest from the biogenetic point of view, but also may provide a clue to the best method of destroying the insects.

Since repeated crystallisation caused but little alteration in its melting point, the wax is probably a single substance or a mixture of closely related compounds. On hydrolysis, approximately equal amounts of an acid and of an alcohol were formed, analyses and melting points of which indicated their identity as " cerotic " acid, $C_{26}H_{52}O_2$, and " ceryl " alcohol, $C_{26}H_{54}O$, respectively. Confirmation of this was obtained by oxidising the alcohol with chromic acid, whereby it was converted into an acid closely similar to that formed by the hydrolysis of the wax.

It has recently been shown (Piper, Chibnall, and Williams, *Biochem. J.*, 1934, **28**, 2175; Chibnall, Piper, Pollard, Williams, and Sahai, *ibid.*, p. 2189) that in general the acids and alcohols derived from natural waxes are mixtures; and in particular that " cerotic " acid and " ceryl " alcohol from these sources are mixtures having an approximate mean chain length of 26 carbon atoms.

By applying the data contained in the first paper mentioned above to the products isolated from *Cryptococcus fagi* wax, an insight has been gained into its approximate composition. The alcoholic component gave an acetate, m. p. 58·7—59·1°, indicating a mean chain length of 25·6. The m. p. of the free alcohol (77·5—78·0°) showed that a mixture of this mean composition must be at least ternary: and both this m. p. and that of the derived acid are satisfied by a mixture of about 80% of the C_{26} compound with 20% of a mixture of the C_{24} and C_{28} compounds, the former predominating. The acid component, m. p. 81·1—82·0°, gives an ethyl ester, m. p. 60·4—60·8°, and is therefore a ternary mixture containing some 70—80% of the C_{26} acid, together with 30—20% of the C_{24} and C_{28} acids.

The wax of *Cryptococcus fagi* consists, therefore, of a mixture of esters of the C_{24}, C_{26}, and C_{28} acids and alcohols, in which the C_{26} compounds largely predominate.

The suggestion of Chibnall, Latner, Williams, and Ayre (*Biochem. J.*, 1934, **28**, 313) that a second hydrolysis with sodium ethoxide is essential if the alcoholic constituent is to be obtained pure, was fully confirmed.

Experimental.

Isolation of the Wax.—The felted mixture of insects and wax (96 g.) was scraped off the trunks of affected beech trees, and the wax dissolved out by several extractions with hot chloroform. The filtered extracts were united and evaporated to 250 c.c., and an equal volume of hot ethyl alcohol added. Crystallisation began at once, and, after cooling, the wax was collected, washed with alcohol, and dried in the air. Yield 38 g., m. p. 80·2—81·3°. For purification it was crystallised several times from chloroform (charcoal being used in the first crystallisation), and finally from chloroform–alcohol, the latter being in excess. It separated from this mixture in colourless shining plates, m. p. 81·0—81·5°, particularly suitable for filtering and drying (Found: C, 81·5; H, 13·8. $C_{52}H_{104}O_2$ requires C, 82·0; H, 13·8%).

Hydrolysis of the Wax.—The purified wax (7 g.), caustic potash (7 g.), and ethyl alcohol (150 c.c.) were refluxed together for 17 hours. Calcium chloride (10 g.) in alcohol (70 c.c.) was then added, and the heating continued for a further 2 hours. The calcium salts were filtered off hot, washed with boiling alcohol, again boiled with alcohol and filtered off hot, and finally boiled with chloroform, filtered, and repeatedly washed with hot chloroform. After drying they were decomposed by boiling for an hour with acetic acid, the solution being filtered, and poured into water. The precipitated acid was crystallised three times from acetic acid (charcoal), from which it separated in feather-like aggregates of long colourless plates, m. p. 81·1—82·0° (Found: C, 79·0, 78·8; H, 13·4, 13·5. Calc. for $C_{26}H_{52}O_2$: C, 78·7; H, 13·2%). The ethyl ester melted at 60·4—60·8°.

The alcoholic filtrates from the calcium salts deposited the crystalline alcohol on cooling. It melted over a considerable range, and could not be purified by repeated crystallisation (Found: C, 80·9, 80·7; H, 14·3, 13·9%). It was therefore further hydrolysed by refluxing with a solution of sodium (2 g.) in alcohol (75 c.c.) for 20 hours. After precipitation of the calcium salts the alcohol was isolated as before and crystallised from acetone (250 c.c.) (charcoal), cooling only to about 25°. The alcohol (2·35 g.) crystallised in rosettes of long glistening

plates, and, after two further crystallisations from the same solvent, melted at 77·5—78·0° (Found : C, 81·5; H, 14·2. Calc. for $C_{26}H_{54}O$: C, 81·6; H, 14·2%). The acetate, prepared by boiling the alcohol with pure acetic anhydride for 4 hours, melted at 58·7—59·1°.

Oxidation of the Alcohol to the Related Acid.—A solution of the alcohol (191 mg.) in acetic acid (30 c.c., purified by distillation over chromic acid) was treated at the b. p. during 10 minutes with a solution of potassium dichromate (98 mg.) in 90% acetic acid (10 c.c.), and constantly shaken. After being kept near the b. p. for 1 hour, the mixture was allowed to cool, and the solid collected. Since the m. p. was not sharp, the acid was converted into its calcium salt, which was purified by extraction with hot alcohol and chloroform. The regenerated acid, after crystallisation from acetic acid, melted at 81·5—82·3°, and the mixture with an equal amount of the acid prepared by the hydrolysis of the wax had m. p. 81·2—82·0°.

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