CONVERSION OF CHOLESTANOL TO Δ^7-CHOLESTENOL BY THE GERMAN COCKROACH

Spiro J. Louloudes, Malcolm J. Thompson, R. E. Monroe, and W. E. Robbins

Entomology Research Division
Agricultural Research Service
United States Department of Agriculture
Beltsville, Maryland

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In a study concerned with the utilization and metabolism of dietary 4-C^{14}-cholestanol by the German cockroach (Blattella germanica (L.)), no conversion of cholestanol to cholesterol was detected (Louloudes et al., unpublished data). However, as much as half of the total C^{14} sterols isolated from the roaches was a cholestanol metabolite, which behaved as Δ^7-cholestenol when either the free sterol or its acetate was analyzed by gas-liquid chromatography and reverse isotope dilution. This metabolite was also found to be formed from C^{14}-cholestanol in roaches reared under aseptic conditions, according to the procedure of Clayton (1959).

In order to obtain sufficient material to confirm the identity of this metabolite, large numbers of roaches were reared on a synthetic diet (Noland, 1954) containing 0.2% cholestanol and subminimal quantities of cholesterol. The cholestanol used in these studies was purified several times, as described by Bruce and Ralls (1943), and by chromatography on alumina. This compound had a melting point of 143°-4° C. and was found to be pure by gas-liquid chromatographic analysis.

The roaches (855), weighing 30 grams, were extracted according to previously reported methods (Kaplanis, 1961), to give 878 mg. of lipid residue which yielded 155 mg. of nonsaponifiable material. An ultraviolet spectrophotometric analysis of this material did not show the characteristic absorption bands exhibited by the $\Delta^{5,7}$ diene system. However, weak absorption bands were observed at 232 and 280 mμ. Chromatography of this

material on alumina (Woelm, Neutral Grade, Activity I) gave 25 mg. of monohydroxylated alcohols. Resolution of the sterol mixture was accomplished by preparing the p-phenylazobenzoyl esters and chromatographing them on a silicic acid-celite column, as described by Idler and Baumann (1952).

The azoate esters after developing into two major and two minor zones, were mechanically separated and saponified. Reading from the bottom of the column upward, zones three and four represented trace amounts of material and were not further characterized. Zone one was identified as cholestanol by gas-liquid chromatography, infrared spectroscopy, and melting point. Zone two, representing approximately 50% of the mixture, was identified as Δ^7-cholestenol by comparison with an authentic sample. The melting point of the free sterol (120.5°-122° C.) was not depressed on

TABLE I

Comparison of gas chromatographic relative retention times of the roach sterol and acetate with Δ^7-cholestenol and Δ^7-cholestenol acetate

Compound	Relative retention time[a]		
	NGS[b]	QF-1[c]	SE-30[d]
Δ^7-Cholestenol	7.96	3.69	2.02
Roach sterol	7.89	3.71	2.03
Δ^7-Cholestenol acetate	7.06	6.09	2.89
Roach sterol acetate[e]	7.06	6.01	2.90

[a]/Relative to cholestane.

[b]/Column 6 ft. x 5 mm. ID, 0.75% neopentyl glycol succinate on 100-140 mesh. Gas-chrom P, 25 psi, 215° C., cholestane time 2.65 min.

[c]/Column 6 ft. x 5 mm. ID, 1% QF-1 (10,000 CS) on 100-140 mesh. Gas-chrom P, 28 psi, 200° C., cholestane time 6.85 min.

[d]/Column 6 ft. x 5 mm. ID, 0.75% SE-30 on 100-140 mesh. Gas-chrom P, 12 psi, 236° C., cholestane time 7.45 min.

[e]/Average of two determinations.

mixing with authentic Δ^7-cholestenol. Its infrared spectrum was identical with that of Δ^7-cholestenol. The acetate was prepared and was identical in melting point and infrared spectrum with Δ^7-cholestenol acetate. The free sterol and its acetate from the roach were further analyzed by three gas-liquid chromatographic systems (VandenHeuvel et al., 1961). Table I summarizes the relative retention times of the roach sterol and its acetate as compared with authentic standards.

The significance of this conversion (of a stanol to a Δ^7-stenol) and the accumulation and function of the metabolite in this insect is not presently understood. However, it bears an analogy to the dehydrogenation of Δ^5-sterols to form 5,7-dienes, a conversion that has been reported for the German cockroach and certain other insects (Beck and Kapadia, 1957; Kaplanis et al., 1960; Robbins et al., 1961).

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